Photosynthetic Gas Exchange in the Closed Ecosystem for Space

Part I. Pilot Photosynthetic Gas Exchange Studies

NASA Contract No. NASW-95 1960-61

E.A. Zuraw
B.J. Weissman
R.P. Casey
V.A. Speziali
T.A. Adamson

GENERAL DYNAMICS CORPORATION
Electric Boat Division
Groton, Connecticut

Research and Development Department

Examined: R. J. Benoit
R. J. Benoit
Supervisor, Biochemical Processes

Approved: A. Bialecki
A. Bialecki
Head, Chemical Engineering Section

U411-61-131
August 1961

FOREWORD

This three-part report describes a multiphase program carried out by General Dynamics Corporation's Electric Boat Division under Contract NASW-95, "Photosynthetic Gas Exchanger," issued by the National Aeronautics and Space Administration, Washington, D.C. Phase I (1959-1960) studies were described in a final report entitled, "Photosynthetic Gas Exchange in the Closed Ecosystem for Space," SPD60-085, 1960.

The final report for Phase II (1960-1961) is divided into three parts: Part I, Pilot Photosynthetic Gas Exchanger Studies; Part II, Studies on the Growth of Thermophilic Chlorella 71105; and Part III, Algae Screening and Mutation Studies. The objectives and organization of each part are detailed below.

The objectives of Part I studies were: 1) the design and fabrication of a pilot-plant photosynthetic gas exchange system, 2) operation of the system to establish the gas exchange capacity of the PGE in relation to light intensity and intermittency, and 3) to review the use of algae as food and in waste treatment for space travel.

Part I of the final report is composed of seven sections: Section I, Introduction; Section II, Design; Section III, Factorial Experiments; Section IV, Chemical Analyses; Section V, Discussion; Section VI, Conclusions, and Section VII, Appendices. Section I, the introduction, briefly reviews the work and results of the Phase I program and the experimental program for Phase II. The photosynthetic gas exchange system designed and fabricated for Phase II is described in Section II. Section III discusses the experimental design, experimental procedure, and results obtained from operation of the pilot photosynthetic gas exchanger. Chemical analyses of algae produced during optimization studies are treated in Section IV. The results of the optimization studies and progress in photosynthetic gas exchange research are discussed in Section V. Conclusions are given in Section VI. In Section VII are drawings, calculations, and summary data. Comprehensive reviews of the literature on the use of algae as food and in waste treatment are given as Appendices A-1 and A-2.

The objectives of Part II, "Studies on Growth of Thermophilic Chlorella 71105" were: 1) optimization of carbon dioxide concentration, dilution rate, and culture volume in bench-scale culture units, 2) the re-use of algal nutrient medium, 3) determination of the optimal composition of the nutrient medium, 4) determination of the effect of pH and growth inhibitors on algal growth and 5) studies on the storage stability of Chlorella 71105.

Part II is composed of six sections: Section I, Introduction; Section II, Experimental Program; Section III, Materials and Methods; Section IV, Results and Discussion; Section V, Conclusions and Recommendations; and Section VI, Appendices. Section I briefly discusses the propriety of

conducting studies in small-scale culture units. Section II describes the experiments conducted in the bench-scale units and in test tubes. The equipment and methodology used in the experimentation is discussed in Section III. Section IV gives the results of studies with unreplenished nutrient medium, a factorial series of experiments on growth parameters, and test-tube studies. Conclusions and recommendations are given in Section V. Detailed data, calculations, etc. are relegated to appendices, Section VI.

Part III, "Algae Screening and Mutation Studies," is divided into two major sections. Section I describes algal screening studies on soil samples obtained throughout the world; Section II describes the effects of ultraviolet radiation on Chlorella 71105.

The people participating in the Phase II experimental program are presented below:

Mr. Barry Weissman	Chemical Engineer	Pilot PGE operations, data reduction and calculations, light measurements
Mr. Harold Wallman	Chemical Engineer (Supervisor)	Equipment design
Mr. Raymond F. Haworth	Mechanical Engineer (Supervisor)	"
Mr. Victor A. Speziali	Mechanical Engineer	"
Mr. Russell Nickerson	Instrumentation Engineer	Instrumentation
Miss Thelma E. Adamson	Scientist	Biochemical analyses
Mr. Richard P. Casey	Scientist	Literature reviews
Mr. Donald E. Leone	Scientist	Bench-scale studies
Mr. Norman A. Richards	Scientist	Algae screening and mutation studies
Dr. Edward A. Zuraw	Scientist	Experimental design and supervision of the experimental program
Mr. John Sorenson	Technical Aide	Pilot PGE operation
Mr. George Erkan	" "	" " "
Mr. Aime Savard	" "	" " "
Mr. Albert Stover	" "	Algae screening and mutation studies

Dr. Richard J. Benoit, supervisor of the Biochemical Processes Group was invaluable throughout the experimental program as well as during preparation of the final report for stimulating discussions, helpful advice, and constructive criticism. He was also instrumental in the organization and integration of the various parts of the report.

Acknowledgement is extended to Mr. Adolph Bialecki, Head of the Chemical Engineering Section, for advice and criticism throughout the experimental program and review of the final report.

The assistance of the following people is also gratefully acknowledged. Dr. Allen Rabe, Mr. Barry Weissman, and Mr. Hancock Chau, are responsible for derivation of equations for describing light distribution within a PGE. Dr. D. Edward Nichols was helpful in consultations concerning experimental design and analysis of results. Mr. Thomas Bolles assisted with gas chromatographic analysis. Mr. Paul Parella was invaluable in expediting and purchasing materials.

Page intentionally left blank

ABSTRACT

A pilot-scale algal photosynthetic gas exchanger (PGE) was designed, fabricated, and operated in a simulated closed gas cycle. A factorial experimental design was used to determine the effect of light input and agitation rate on PGE performance with Chlorella pyrenoidosa 7-11-05 under equilibrium or steady-state conditions. Material balances were calculated for evaluation of process control and data reliability. It was established that light input significantly affected algae production, equilibrium culture density, gas exchange, and algal composition. The effects of the particular agitation rates in this apparatus (or interactions between light input and agitation) were found to be negligible. The highest rate of algae production (4.02 gms/hr), equilibrium culture density (805 mg/L), O_2 production (0.138 SCFH), CO_2 consumption (0.112 SCFH), occurred at the highest power input of 7.5 KW. Thus, the gas exchange capacity of the PGE was equivalent to the respiratory requirements of 0.14 man.

Chemical analyses of the algae showed that the chlorophyll, nitrogen, and carbon content of the algae were lowest at the highest light input; yet, the highest light input resulted in the highest rate of algae and O_2 production. Theoretical gas exchange and derived values such as carbohydrate, protein, and lipid were calculated from the elemental analyses.

Theoretical and measured gas exchange agreed within 20%; calculated biochemical composition (protein, fat, lipid) agreed well with values reported by other workers. An average equivalence of 1.03 liters of oxygen per gram (dry) algae, and an assimilatory quotient (AQ) of 0.83 were determined.

A mathematical model was formulated describing the light intensity distribution within the culture suspension at various light inputs, suspension depths, and culture densities.

Reviews of recent literature on algae as food and algae in waste treatment are presented.

Page intentionally left blank

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont.)

TABLE OF CONTENTS (Cont.)

LIST OF TABLES

LIST OF TABLES (Cont.)

LIST OF FIGURES

SECTION I

INTRODUCTION

Page intentionally left blank

SECTION I

INTRODUCTION

The concept of a closed ecosystem for space requires the ultimate integration of a wide variety of interdependent factors. A general discussion of the known significant factors in the production of a closed photosynthetic gas exchange system was presented by Zuraw et al, "Photosynthetic Gas Exchange in the Closed Ecosystem for Space", (1960) and in a proposal submitted to NASA entitled, "An Engineering Approach for a Closed-Cycle Photosynthetic System" (1959).

For the past two years, research effort at Electric Boat Division, under the auspices of NASA, has emphasized engineering studies on one of the problems most basic to such a system, the provision of a physiological atmosphere by means of photosynthesis. During this period, two decidedly different pilot-scale experimental photosynthetic gas exchangers were designed and fabricated. Experimental test programs with the units, oriented towards defining engineering design, and operational problems peculiar to photosynthetic gas exchanger, were conducted. Although all engineering design and operational problems of a photosynthetic gas exchanger (PGE) were not resolved, important information was derived from completed studies. In addition to the pilot studies, supporting experimental investigations were conducted on allied aspects of photosynthetic gas exchange in a closed ecological system.

PHASE I RESEARCH PROGRAM (1959-1960)

During Phase I (1959-1960), a pilot-scale photosynthetic gas exchanger was designed, fabricated, and tested. The gas exchange capacity of the pilot PGE was measured under conditions of: 1) closed cycle, coupled with chemical oxygen absorbers; 2) closed cycle, short term; 3) open system, single pass; and 4) closed cycle, coupled with a primate chamber. In support of these pilot-scale studies, a series of other specific small-scale studies were carried out. The supporting studies included investigations of 1) variations in nutrient medium and their effects on algal growth, 2) recycled medium, fecal ash, urine, and urinary components as algal nutrients, 3) transfer, absorption, and concentration effect of carbon dioxide, 4) other thermophilic algae as photosynthetic agents, 5) bacterial contaminants and their effects on gas exchange, and 6) design and operation parameters for PGE units.

From Phase I investigations, the following information was obtained. Operation of the 60-liter, pilot PGE established that mass cultures of Chlorella pyrenoidosa 7-11-05* can be maintained in a state of rapid metabolic activity for a period of at least six months. Two

*A thermophilic alga isolated by Sorokin and Myers (1953). For convenience, we refer to the strain as Chlorella 71105.

primary factors found to govern algal production and the concomitant rate of gas exchange were dilution factor (the ratio of nutrient medium feed rate to total culture volume), and the ratio of the light surface area to culture volume. Other factors, such as nutrient composition and carbon dioxide level, had to be considered as secondary. With the Phase I pilot-scale PGE, the maximum amount of oxygen produced was 5.2×10^{-3} lbs/hr at an algal production rate of 14.8×10^{-3} lbs/hr (wet weight) and a culture density of 0.31% (packed cell volume). Carbon dioxide was simultaneously consumed at a rate of 4.7×10^{-3} lbs/hr. The gas exchange capacity of the pilot PGE was shown to be more than adequate to provide for the gas exchange requirements of a 3-pound primate for 50-hour periods. Supporting studies showed that the nutrient medium supernatant in which Chlorella 71105 was grown may be re-used provided that the nutrients consumed are replaced. Although diluted urine supported good growth of the organism for short periods, long term tests showed that diluted raw urine was not entirely suitable. Growth tests with incinerated feces were inconclusive, although fecal ash is known to contain the elements required for algal growth. Various membranes were evaluated as gas-permeable, liquid-impermeable barriers; permeabilities of 0.2 to 2.0×10^{-3} SCFH of CO_2 per $ft^2/mil/\Delta psi$ were obtained. Manometric studies on bacteria isolated from the pilot PGE indicated that bacterial contaminants do not significantly interfere with the apparent photosynthetic capabilities of Chlorella.

The significant difficulties encountered in the operation of the PGE were largely mechanical and instrumental. The algae did adhere to surfaces not subjected to adequate liquid turbulence; and foaming did occur, but was controlled with a silicone-type antifoam. It also proved difficult to match the assimilatory quotient of the algal suspension to the respiratory quotient of the monkey whose gas exchange requirements were being provided for by the photosynthesizing culture. The major problem in experimentation centered about the detection and prevention of leaks in the closed-cycle gas system. Some analytical measurements were difficult to make with the precision and reliability required for adequate control of the closed-cycle system. However, it was considered that these difficulties could be overcome by the proper choice of materials of construction, auxiliary equipment, and fabrication techniques in future work.

PHASE II RESEARCH PROGRAM (1960-1961)

During Phase II (1960-1961) a new experimental pilot-scale PGE was designed, fabricated, and operated under simulated closed-gas-cycle conditions.

A factorial experimental program was conducted in an attempt to optimize the parameters of light input and agitation. The gas exchange capacity of the system was determined in relation to different levels of light intensity and agitation by gas exchange measurements and chemical analyses of the algae produced.

In addition to the pilot PGE studies, literature surveys were conducted on the use of algae as food and in waste processing in a closed ecosystem.

The pilot-scale PGE program in Phase II, as in Phase I, was supported by several specific studies on a smaller scale. The small-scale studies were conducted to help define a program for pilot-scale studies, and to limit ranges of parameters to be studied. The results of the subsidiary programs have been submitted as Parts II and III of this final report. Part II, "Studies on the Growth of Thermophilic Chlorella 71105", describes studies on the re-use of nutrient medium and the effect of nutrient feed rate, CO_2 concentration, culture volume, and composition of the growth medium on growth of Chlorella 71105. Part III "Algal Screening and Mutation Studies", reviews the screening of 140 soil samples for algae having growth rates superior to Chlorella 71105 and describes attempts at inducing genetic mutations with useful characteristics.

Page intentionally left blank

SECTION II

DESIGN

Page intentionally left blank

SECTION II

DESIGN

BACKGROUND

The completely closed ecological system in which an algal culture supports the human physiological activity necessarily involves a complex working system. Although details are extensive, the present state of technology does not permit any limited program to engage the problem with hopes of easy success. For this reason, the approach has been to divide the system into its major areas and to investigate them individually. The experimental photosynthetic gas exchange system described in this section has been designed with these considerations in mind; it is essentially a research pilot plant.

SYSTEM PARAMETERS

The general wear-and-tear of the Phase I plastic PGE through continual use and modifications precluded reuse of the system for the Phase II experimental program. Therefore, a new PGE system was designed and built based on the results and experience gained in operating the Phase I system. Our configuration was originally based on a standard length of large-diameter flanged pipe. The lead-time on glass pipe proved to be prohibitively long so we accomodated our design to a readily available glass reactor flask. The design was based on the following considerations:

1. The PGE system should have a high degree of hermetic integrity and provisions for the detection of such leaks as might occur.
2. The PGE system should be automated and instrumented for precise control of the system and ease of operation.
3. The PGE system should be of stainless steel with welded construction where feasible.
4. The PGE should provide versatility in lighting arrangement.
5. The light transmitting medium should be glass rather than plastic.
6. The PGE should provide mechanical agitation of the algal suspension.

For design purposes, the following characteristics of the thermophilic alga, Chlorella 71105, were assumed:

1. Chlorella cells double every four or less hours.

2. Dry algal cells contain 50% carbon by weight.

3. Fresh algal cells contain 25% dry material.

4. Oxygen production and the formation of new algal material can be approximated by the equation:

$$6\ CO_2 + 6\ H_2O \longrightarrow C_6\ H_{12}\ O_6 + 6O_2$$

5. The heat of formation of algae is approximately 112 kcal/gm mol of CO_2 consumed or O_2 produced.

6. Chlorella 71105 will grow well with urea as the exclusive nitrogen source and will carry out photosynthesis with an assimilatory quotient (AQ) between 0.80 and 0.90.

7. The algae have an optimum growth temperature of 102°F and the culture temperature must not exceed 104°F.

8. The algae can be cultured under non-sterile conditions. However, the equipment should be designed so as to minimize the accumulation of dead or moribund algal cells.

9. The algae reach light saturation at about 1600 foot candles, and in dense, turbulent cultures do not show solarization effects up to an intensity of about 12,000 foot candles.

Since the primary purpose of the PGE was to obtain operational and design data, the possible occurrence of trace gases, such as methane and hydrogen sulfide, and other minor contaminants did not influence equipment design. Furthermore, factors pertaining to human protection and comfort (e.g., radiation, acceleration, noise, zero gravity) also did not appreciably influence the design of the system.

SYSTEM DESIGN

The above factors were considered in a design approach in which flow diagrams, flow sheet calculations, and equipment drawings and sketches were prepared. The size of the component parts for the system were calculated, and equipment and material lists were prepared from the drawings and tables.

The major subsystems included the photosynthetic gas exchanger, a gas circulation loop, a nutrient medium feed system, a suspension over-flow system, and a gas analysis system. All components, sub-systems, and circulation loops were designed in an open, spread-out arrangement, with no special concern for compactness or miniaturization. This open arrangement provided interchangeability of components, ease of maintenance, and experimental versatility.

THE PHOTOSYNTHETIC GAS EXCHANGER

The PGE consisted of two concentric cylindrical annuli. The outer annulus, containing cooling water, was bounded by two stainless steel

shells. The inner annulus, containing the algal suspension, was bounded by the inner steel shell and a dome-shaped glass vessel. See Figure 1a, 1c, and 1d. The outer annulus was cross baffled and provided with a cooling water inlet and outlet. Water was circulated through this jacket to maintain a fixed suspension temperature. A gas manifold, situated at the bottom of the jacket, was of toroidal shape with a rectangular cross section. The inner wall of the manifold was penetrated by sixty evenly spaced holes (0.0465-in), which allowed the system gas to pass into the algal suspension. Gas entering the suspension from the manifold bubbled through the culture to the head-space above the glass dome.

The algal suspension was stirred with an agitator (Figure 1b). The agitator shaft passed through the top of the unit and was sealed by means of a manometric well, which constituted a dynamic gas seal. The agitator was driven by an electric motor, mounted on the top of the unit, through a crank and rocker linkage with provisions for speed and stroke variation.

In addition to the manometric shaft seal mentioned, the PGE had a second major seal, located at the bottom where the glass dome mated against a flange welded to the two steel cylinders. A large O-ring sealed the glass to the flange. The glass dome was backed up by a steel bolting ring covered with a thick rubber pad to prevent chipping or cracking the glass. To prevent the agitator from hitting the glass dome, a plastic bumper was attached to the agitator periphery, limiting its radial movement.

A lighting harness designed to hold from one to ten 1500-watt incandescent lamps in a variety of geometrical arrays was positioned within the dome. The lamps were cooled by a ducted blower which forced room air up into the dome around the lamps. The temperature of the inner surface of the dome was monitored by thermocouples.

The entire PGE was fabricated using only types 304 and 316 stainless steel, glass, silicone rubber, teflon, nylon, and plexiglas where contact with the algal culture or medium was made. However, piping and some components not in contact with the suspension were fabricated from other materials such as carbon steel, copper, and brass.

Details of the PGE design may be found in the Assembly Drawing in Appendix E.

AUXILIARY EQUIPMENT

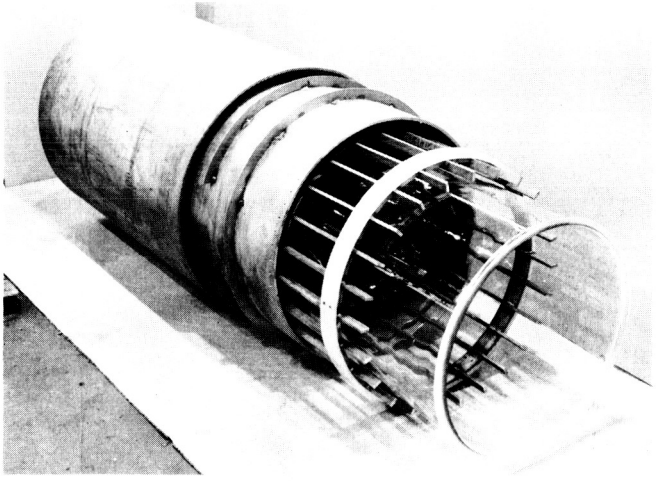
In addition to the PGE itself, operation of the system was also dependent on several subsystems and auxiliary components - the gas dehumidifier, gas surge tank, gas pump, the nutrient medium feed system, lighting system, lamp cooling system, and the instrumentation and control system.

A gas dehumidifier (condenser) was located in the gas exit line to eliminate condensation in the gas surge tank and gas analysis equipment. The condensor was a jacketed 304 stainless steel pipe with

a. COOLING WATER INNER JACKET

b. AGITATOR

c. GLASS DOME



d. ASSEMBLY (EXPLODED VIEW)

FIGURE 1 PGE INTERNAL COMPONENTS

cooling water circulating counterwise in the outer jacket.

A 10.55 cubic foot (299-liter) stainless steel cylinder with hemispherical ends served as a gas surge tank to dampen any pressure fluctuations in the gas circuit. It also provided a good gas mixing space for CO_2 and N_2 replenishment.

A size B Leiman Bros. air pump, driven at 800 rpm by a 3/4-hp motor, was used for gas circulation. The pump discharged into a Wilkerson filter to remove any oil in the gas stream. Filter connections were reversed to provide gas flow in the center of the element, through the filter medium, and outward into the glass bowl to the outlet.

The nutrient medium feed system consisted of two 55-gallon stainless steel drums and a piston feed pump. Piping was arranged so that the drums could be alternately cleaned, flushed, and refilled with fresh nutrient during continuous operation. The nutrient feed pump, a BIF model 1106 with a capacity of 3.2 gallons per hour, was driven through a speed reducer by a 1/4-hp motor.

The lighting harness was designed to position one to ten high-intensity incandescent lamps in a vertical position within the dome. The harness consisted of a base plate with interchangeable vertical rods on which lamp retaining tabs were positioned. Scribe marks on the vertical rods, lamp retaining tabs, base plate, and base of the PGE fixed the position of the lamps during the experimental runs. Heat generated by the lamps was removed from the glass dome by a 1/2-hp Coppus blower, type 175, with a capacity of 1500 CFM.

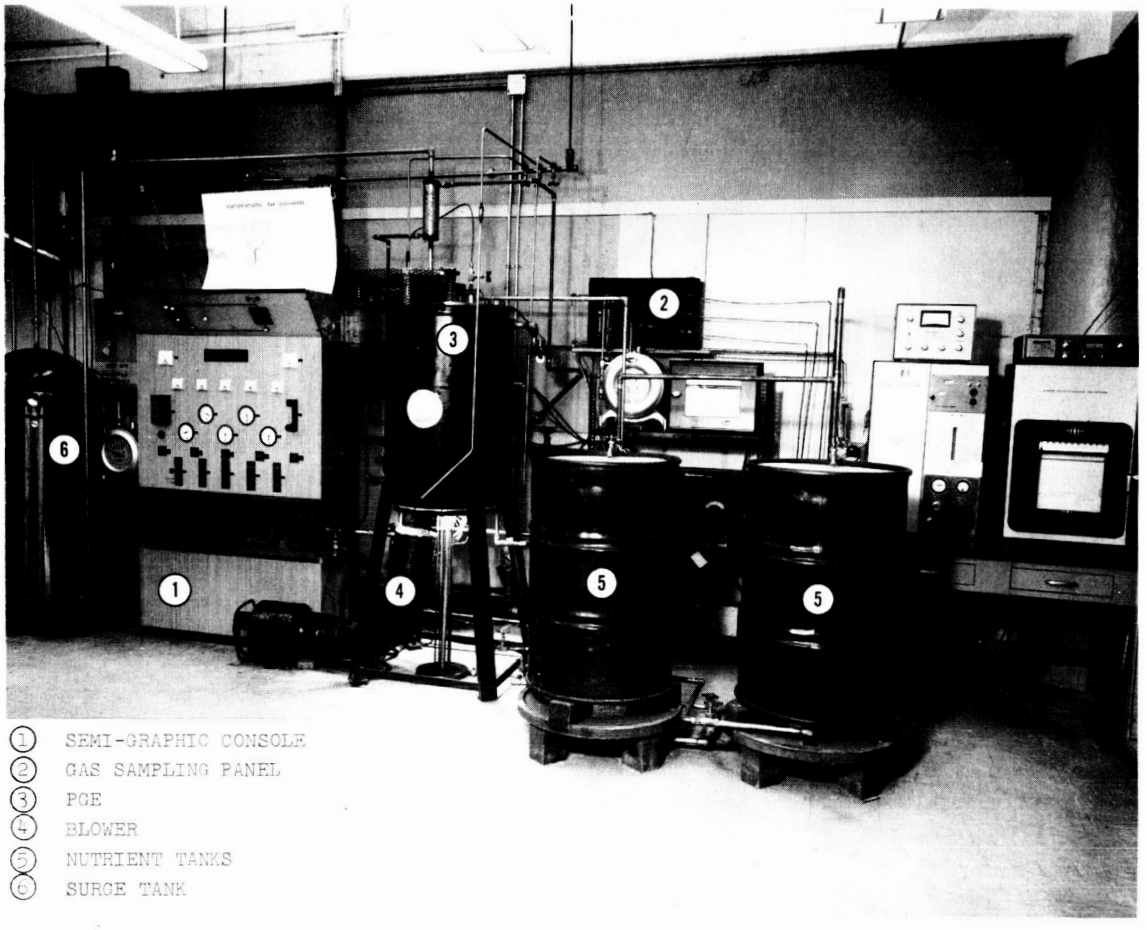
INSTRUMENTATION AND CONTROL

The centralized control system consisted of a semi-graphic console and a gas sampling panel (see Figure 2). System pressures and temperatures, gas analyses, gas flow rates, lamp voltages, and alarm conditions were displayed on the console. The on-off status of pumps, drains, and gas supply pressures were also shown.

Both manual and automatic controls were provided for regulating lamp voltages, gas flows, pump operation, etc. Interlock (automatic, overriding safety) controls were used for regulating algal temperatures, cooling water flow, air pump operation, and lamp voltage (see Appendix B-2b, Drawing Nos. 3063 and 3065).

At the gas sampling panel, automatic control of the system pressure was provided by means of a back-pressure control valve on the gas vent line. Manual controls were also provided for standardization of the gas analyzers. A rotameter indicated the flow rate of vented gas.

A number of automatic controls and interlocks were provided. The addition of CO_2 and N_2 to the system was controlled by Moore Products purge gas flow controllers, Model 63BD. Indicating rotameters with the following ranges were used:



① SEMI-GRAPHIC CONSOLE
② GAS SAMPLING PANEL
③ PGE
④ BLOWER
⑤ NUTRIENT TANKS
⑥ SURGE TANK

FIGURE 2 THE PHOTOSYNTHETIC GAS EXCHANGE SYSTEM

CO_2	0.2 to 2.5 SCFH
N_2	0.02 to 0.25 SCFH.

These controllers regulated the differential pressures across adjustable needle valves.

The gas venting from the system was controlled by a Moore Products back-pressure regulator, Model 43R, operated on an absolute pressure basis at 15 psia.

The temperature of the algal suspension was controlled by the on-off action of the cooling water flow. A Fenwal Model 17001-12 "finger probe" with adjustable bi-metallic contact action regulated the water flow.

Two Champion Model J-14-64C-3 level probes were used in a differential gap control circuit for liquid level control. A solenoid valve in the culture drain line, activated by the two probes, maintained the culture volume between 65.5 and 66.5 liters.

Lamp voltage was controlled by five autotransformers through a relay switch. The relay was maintained closed by four permissive signals:

Culture level
Culture temperature
Cooling water pressure
Circulating gas flow

On power failure or manual shutdown, all circuits dropped out. Manual re-starting of all circuits was required, to ensure safe operation (see Appendix B-2b, Drawing 3064).

PHOTOSYNTHETIC GAS EXCHANGE SYSTEM OPERATION

The PGE and auxiliary equipment are sketched in Figure 3, and the system process is given in a flow diagram, Figure 4. Specifications for the PGE and its auxiliary equipment can be found in Appendix B-1.

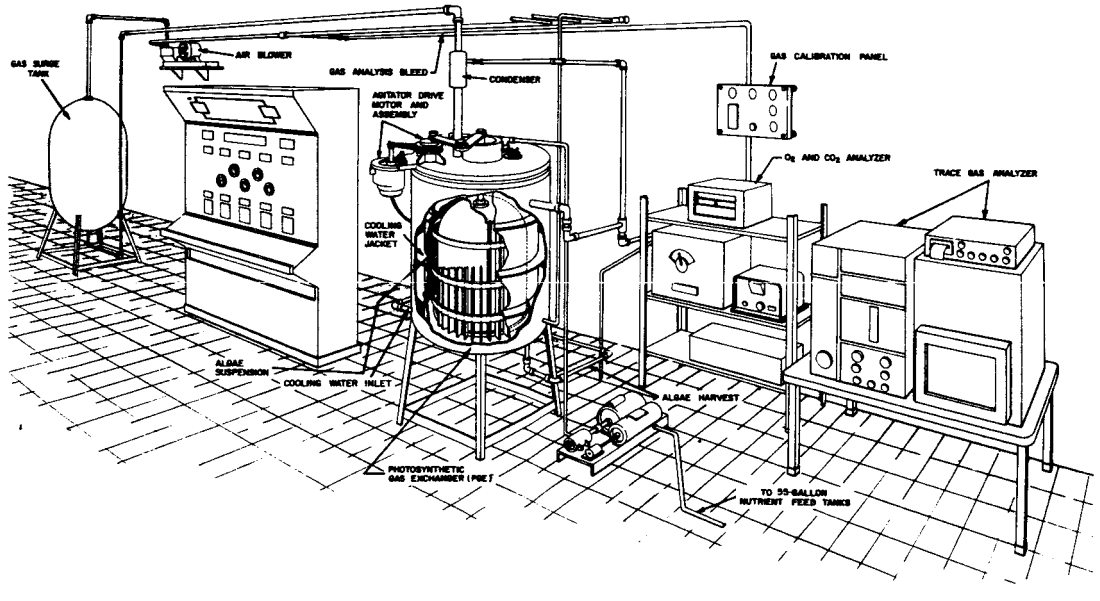


FIGURE 3 THE PHOTOSYNTHETIC GAS EXCHANGE SYSTEM

In the closed-loop gas circuit, CO_2-rich air was pumped to the PGE through the gas manifold at the base of the unit. From there it was forced into the algal suspension through the manifold orifices. The algal suspension was continuously agitated to provide uniform gas distribution and to prevent the algae from settling. The head-space gas leaving the PGE passed through the gas dehumidifier into the gas surge tank where it was mixed with metered supplements of CO_2 and N_2. The gas mixture was drawn from the surge tank into the circulating pump and recycled.

The total gas flow and the composition of the gas entering the PGE were both fixed. Since algae consume CO_2 while producing O_2, the composition of the gas stream leaving the PGE differed from that entering the unit. To maintain the fixed gas concentrations, controlled amounts of CO_2 and N_2 were metered into the surge tank. The introduction of supplementary CO_2 and N_2 into the gas stream increased the total amount of gas, thereby increasing the gas pressure. To maintain a fixed pressure, gas was vented from the system downstream of the circulating pump by a pressure regulating valve preset at 15.0 psia. The vented gas passed through a calibrated rotameter and a series of analyzers (paramagnetic oxygen analyzer, infra-red carbon dioxide analyser, and vapor fractometer), thus providing continuous gas monitoring. From the gas analyzers, the gas stream was finally vented to the atmosphere.

CO_2 and N_2 were admitted to the surge tank through calibrated rotameters. Since the gas composition was continuously recorded, the amounts of

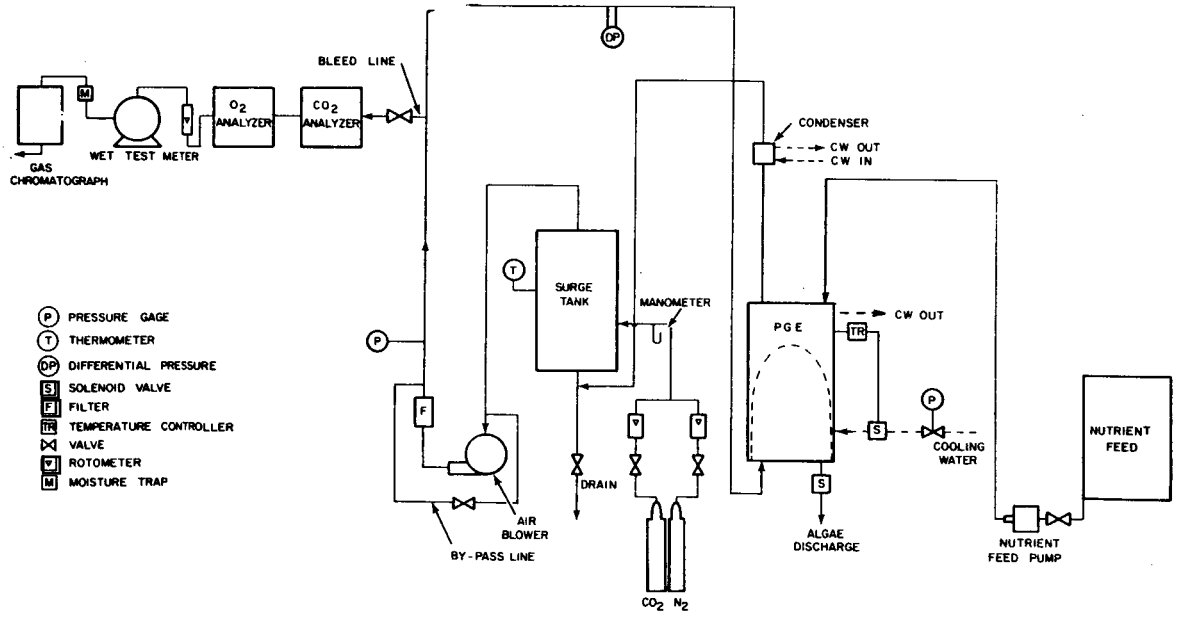


FIGURE 4 SCHEMATIC DIAGRAM OF THE PHOTOSYNTHETIC GAS EXCHANGE SYSTEM

CO_2 and N_2 required to maintain a constant composition were determined simply by adjusting the rotameters until CO_2 and O_2 concentrations in the vent line remained constant. This method required intermittant adjustment of the CO_2 and N_2 inlet rotameters until a steady-state was achieved; i.e., until the culture reached an equilibrium gas exchange rate.

With the exception of the manually operated gas composition control system, the remainder of the operation was automatic. Algal suspension temperature, nutrient medium feed, algal suspension harvest, and emergency shutdown were all automatically controlled. However, manual overrides were provided in each case.

The algal suspension temperature was controlled at $102^{\circ}F \pm 0.5^{\circ}F$ by regulating the flow of cooling water. The flow was controlled by a CRES bimetallic thermoswitch immersed in the suspension, which activated a solenoid valve in the cooling water line. A second thermoswitch was used to shut down the system automatically if the culture temperature exceeded $104^{\circ}F$.

A continuous flow of nutrient medium into the PGE was maintained by means of the calibrated nutrient feed pump. The harvesting of algae was controlled by a pair of liquid level probes. The two probes were connected to a solenoid valve in the drain (harvest) line. When the liquid level in the PGE contacted the upper probe, the solenoid valve was opened, allowing the culture to drain. When the level within the unit reached the tip of the lower switch probe, the valve closed.

The third probe extended lower than the level-control probes. If the culture level dropped below the probe, the entire system was shut down.

To summarize the fail-safe provisions, power to the lamps was shut down automatically by any one or a combination of the following conditions:

1. Drop of pressure in the cooling water line below 25 psig.
2. Rise of algal suspension temperature above $104^{\circ}F$.
3. Fall of suspension level.
4. Failure of the gas circulating pump as detected by a differential pressure switch set at 1/2 psig.

An integrated control panel was equipped with both visual and audio alarms to warn operators of malfunction or failure of components. For example, if the pressure in the CO_2 or N_2 supply cylinders were to drop below 15 psig, an alarm sounded, warning the operator. Each component was represented on the control panel by a code signal light. Faulty operation or failure triggered the audio alarm and flashed the appropriate light on the panel. In this manner malfunctions could be both detected and located easily.

A detailed operator's manual was prepared to instruct laboratory personnel in proper operation of the system.

Page intentionally left blank

SECTION III

FACTORIAL EXPERIMENTS

Page intentionally left blank

SECTION III

FACTORIAL EXPERIMENTS

EXPERIMENTAL DESIGN

An outline of the variables studied and controlled in the factorial experiments is presented in Table 1.

Two independent variables, power (light) input and agitation, were chosen for study because of the following considerations:

1. Light is basic to photosynthesis.
2. Algae are light-saturated at low light intensities.
3. The sun provides light of high intensity.
4. Algae can integrate high input intensities if exposed to alternate light and dark periods of proper frequency.
5. PGE designs providing low artificial light intensities are necessarily bulky.
6. PGE designs providing high artificial light intensities may be made more compact.
7. Artificial lighting in general requires a considerable expenditure of energy, most of which is in the form of heat.

Three orthogonally spaced power input levels of 7.50, 5.85, and 4.20 KW, in conjunction with two agitation rates (45 and 90 cycles/min), were studied. The controlled factors included CO_2 and O_2 concentration, culture volume, temperature, nutrient feed rate, nutrient composition, gas pressure, and gas recirculation rate.

The measured dependent variables were: 1) equilibrium culture density, 2) algae production, 3) O_2 production, 4) CO_2 consumption, 5) elemental algal composition, and 6) chlorophyll, moisture, and ash content.

The factorial experimental design consisted of six different combinations of the two independent variables, and therefore required six runs with the PGE. The six runs were made in a random order and were repeated once for a total of 12 runs.

The results were subjected to an analysis of variance. The mean square variance due to each factor (power input and agitation) was determined. Since the levels of power input were orthogonally spaced, it was possible to estimate whether the change in response was due to linear or

quadratic influence. It was also possible to estimate the mean square variance due to interaction and random error.

In the statistical analysis, the component of variance indicated whether there was a significant difference between any pair of the independent variable levels. Furthermore, the magnitude index gave the order of the component according to its contribution to the change of response.

Table 1

Factorial Experimental Program

VARIABLES		LEVELS
Independent	Fixed	
Power Input*		4.20, 5.85, 7.50 KW
Agitation Rate		45, 90 cycles/min
	CO_2	1%
	O_2	21%
	Culture Volume	66 liters
	Temperature	102°F
	Dilution factor	0.07 hr^{-1}
	Nutrient medium	0.5X Standard
	Pressure	15.0 psia
	Gas recirculation	60 CFM
	pH	5.2

*Electrical input to five lamps; the light energy flux at these three electrical levels were: 4.78×10^3, 9.26×10^3, and 15.5×10^3 Kg cal/day or 0.23, 0.45, and 0.75 KW radiant flux in the range 400 to 700 millimicrons.

EXPERIMENTAL PROCEDURE

Before experimental runs were initiated, the PGE system was thoroughly tested for gas leaks by means of static and dynamic pressure tests. The static tests consisted of pressurizing the system with nitrogen to 30 cm (water gage) and noting the pressure change over a few hours. The tolerable pressure loss was set at 0.25 cm H_2O/hr. The dynamic tests involved pressurizing the system to 0.25 psig with Freon, and then turning on the gas pump to circulate the gas mixture throughout the system. An electronic halogen detector was used to test the entire system. Following leak tests, the gas pump was turned on, and the PGE system was vented to remove Freon from the system. The system was considered gas tight if the static tests met the specification set, and if the halogen detector could not detect a leak at its highest sensitivity setting in the dynamic tests.

Inoculum for the PGE was obtained from prior runs or, for Run Nos. 1 and 5, from algae grown in the Phase I pilot PGE (see Zuraw, et al, 1960). The inoculum (30 liters of algal suspension) and the warm nutrient medium (0.5X standard medium at 102°F) were added to the PGE

through a small port at the top until the liquid level just covered the lower liquid level probe. This resulted in a liquid volume of 2.3 ft^3 (66 liters) and a total system gas volume of 12.0 ft^3 (339 liters). The unit was then sealed, and the agitator, nutrient feed pump, and lamps were turned on.

Agitation rate and light input were adjusted to desired levels, and the CO_2 and O_2 concentrations of the circulating air were controlled at 1.00 ± 0.06% and 21.0 ± 0.5%, respectively, by adjusting the CO_2 and N_2 input valves of the rotameters.

The culture was allowed to attain equilibrium under the experimental conditions imposed. Equilibrium of the culture became apparent when culture density measurements and gas exchange rates remained constant. This generally required from 2 to 4 days. Although dependent, independent, and fixed variables were monitored 24 hours a day, 7 days a week, only equilibrium data were used for analysis of results. Equilibrium data were collected by at least 3 different operators for a minimum total period of 24 hours. Gas analysis instrumentation was standardized by each operator once every 8 hours with standard gases.

The various fixed or controlled factors and control limits are presented below.

Suspension volume	66 ± 0.5 liters
System gas flow rate	60 ± 5 CFM
O_2 concentration	21.0 ± 0.5%
CO_2 concentration	1.00 ± 0.06%
Suspension temperature	102 ± 1°F
Pressure, blower output	3.5 ± 0.2 psig
Pressure, blower input	0-20 cm H_2O
Nutrient feed rate	4.9 ± 0.1 liters/hr
Nutrient concentration	0.5X standard medium
pH	5.2 ± 0.1

When any of these factors began to deviate from prescribed levels, immediate manual adjustment was made.

After each run, a new combination of power input and agitation was selected and the system was operated until the new equilibrium was attained.

The glass dome was removed and cleaned after every other run. The culture chamber and agitator were also inspected and cleaned when necessary. Very little algae adhered to the agitator or the stainless steel walls of the culture chamber. Some algae, however, did settle about the bottom periphery of the glass dome at the rubber O-ring. Turbulence was low in this area because of clearances required between the agitator and the dome.

RESULTS

Analysis of Factorial Experiments

The effects of light input and agitation on the various dependent variables found in the factorial experiments are detailed below. In addition, the results of material balances and light intensity measurements are assessed.

The measured responses to the independent variables (power input and agitation) obtained were analyzed by variance analysis on an IBM 704 digital computer. The analyses showed that power input affected the majority of the dependent variables significantly at a 95% confidence level. Agitation had no significant effects in this system.

See Appendix C-1 for a summary of data obtained in the factorial experiments and Appendix C-2 for the results of the analysis of variance of each measured dependent variable.

O_2 Production and CO_2 Consumption

The variance analysis showed that power input significantly affected both O_2 production and CO_2 consumption. Figure 5 shows a smooth curve through the points; the analysis, however, indicated that the relationship did not depart significantly from linearity. Examination of the components of variance on power input revealed that a greater increment of O_2 production and CO_2 consumption resulted by increasing power input from 4.20 to 5.85 KW than from 5.85 to 7.50 KW. This was interpreted as an indication that a limit to O_2 production and CO_2 consumption was being approached with the higher power input and the particular lighting arrangement used.

Agitation did not significantly affect O_2 production or CO_2 consumption over the range tested, nor was interaction between agitation and power input observed. Therefore, a mean value for O_2 production and CO_2 consumption were computed at each power input ignoring agitation rate. The mean values and summary data are given in Table 2. The mean values for O_2 production at power inputs of 4.20, 5.85, and 7.50 KW were 0.081, 0.120, and 0.132 SCFH, respectively. Corresponding mean values for CO_2 consumption were 0.056, 0.097, 0.099 SCFH. A plot of the mean values for O_2 production and CO_2 consumption (Figure 5) confirmed the curvature predicted by the components of variance.

Figure 5 shows that O_2 production and CO_2 consumption increased with increasing power input. The increase in O_2 production obtained by increasing power input from 4.20 to 5.85 KW was 0.039 SCFH while the increase obtained by increasing power input from 5.85 to 7.50 KW was 0.012 SCFH. Corresponding differences observed for CO_2 consumption were 0.041 and 0.002 SCFH.

Mean values for O_2 production and CO_2 consumption were also computed based on algae production and elemental analysis of the algae produced (see Table 3, and Appendix C-3 for sample calculation). A mean O_2 production of 0.036 SCFH/gm algae was calculated for the 12 runs. A corresponding CO_2 consumption value of 0.030 SCFH CO_2/gm algae was obtained. With algae production of 1.60, 2.66, and 3.22 gms/hr at the three power input levels of 2.40, 5.85, and 7.50 KW respectively, calculation of the theoretical amounts of O_2 production and CO_2 consumption resulted in figures of 0.060, 0.098, 0.133 SCFH O_2/hr and 0.048, 0.080, and 0.095 SCFH CO_2/hr, respectively. The theoretical values are plotted with the measured values in Figure 5.

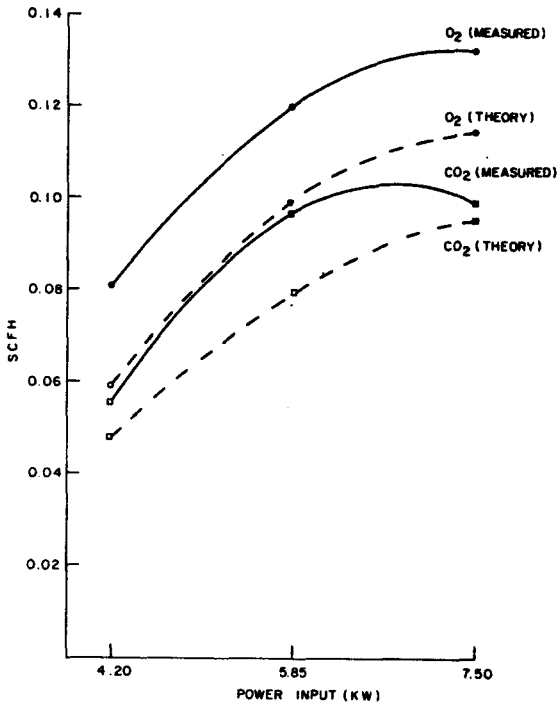


FIGURE 5 EFFECT OF POWER INPUT ON O_2 PRODUCTION AND CO_2 CONSUMPTION

Table 2

Effect of Power Input and Agitation on O_2 Production and CO_2 Consumption

Power Input (KW)	4.20		5.85		7.50	
Agitation Rate (Cycles/min)	45	90	45	90	45	90
O_2 Production (SCFH)	0.067	0.078	0.081	0.113	0.112	0.138
	0.100	0.077	0.147	0.140	0.154	0.124
$\bar{X}$	0.084	0.078	0.114	0.126	0.133	0.131
$\bar{\bar{X}}$	0.081		0.120		0.132	
CO_2 Consumption (SCFH)	0.048	0.052	0.075	0.089	0.081	0.112
	0.067	0.055	0.104	0.119	0.111	0.092
$\bar{X}$	0.058	0.054	0.090	0.104	0.096	0.102
$\bar{\bar{X}}$	0.056		0.097		0.099	

Table 3

O_2 Production and CO_2 Consumption Based on Elemental Analysis of Algae

Run	Light Input (KW)	Agitation Rate (Cycles/min)	O_2 Production per gram algae (L/hr)	(SCFH)	CO_2 Consumption per gram algae (L/hr)	(SCFH)
1	7.50	90	0.97	0.034	0.83	0.029
3	7.50	45	1.08	0.036	0.83	0.029
4	7.50	90	0.98	0.035	0.83	0.029
5	4.20	90	1.05	0.037	0.86	0.030
6	5.85	45	0.98	0.035	0.81	0.029
8	4.20	45	1.08	0.038	0.89	0.031
9	7.50	45	1.05	0.037	0.86	0.030
10	4.20	90	1.05	0.037	0.88	0.031
11	5.85	90	1.04	0.037	0.87	0.031
12	4.20	45	1.04	0.037	0.86	0.030
13	5.85	45	1.09	0.038	0.86	0.030
14	5.85	90	1.05	0.037	0.87	0.031
$\bar{X}$			1.03	0.036	0.86	0.030

Measured and calculated values for O_2 production and CO_2 consumption agreed on the average within 19% and 12%, respectively. Calculated values were consistently lower. It must be remembered that the theoretical values are based on the synoptic equation for photosynthesis which approximates the overall photosynthetic reaction; therefore, the agreement is really quite good. It should also be noted that the best correspondence occurs at the highest gas exchange rate. The data at this point reflect the added precision of gas flow measurements at higher flow rates.

The regression lines of O_2 production and CO_2 consumption on algae production for the 12 runs were computed and plotted with the results shown in Figures 6 and 7.

The equation relating O_2 production to algae production was found to be:

$$\text{SCFH } O_2 = 0.0315 + 0.0317 \text{ (gms algae/hr)}$$

From the slope of this equation, an equivalence of 0.038 SCF O_2/gm/hr, was determined. The equivalence based on elemental analysis mentioned earlier was 0.036 SCF O_2/gm/hr. Thus, the theoretical and measured equivalents agree within 5%.

The equation relating CO_2 consumption to algae production was found to be:

$$\text{SCFH } CO_2 = 0.0172 + 0.0266 \text{ (gms/hr algae)}$$

An equivalence of 0.032 SCH CO_2 consumption/gm/hr was found from the slope of the curve. The theoretical equivalent of 0.030 SCF CO_2/gm/hr, found by elemental analysis, agrees within 6% of the measured value. It appears, therefore, that algae production is a valid measure for predicting gas exchange.

Figures 6 and 7 also show that O_2 production, CO_2 consumption and algae production all increase with increasing power input.

Assimilatory quotients or AQ (mols CO_2 produced divided by mols O_2 consumed) derived from gas measurements ranged from 0.68 to 0.96 with an overall mean of 0.79 for the 12 runs. The broad range in AQ value was interpreted to be due to difficulties in precisely measuring low gas flow rates rather than to some physiological manifestation of the algae. This interpretation is supported if the AQ values based on elemental analyses are examined. These values were very consistent and ranged from 0.82 to 0.85 with an overall mean of 0.83. Of major interest is the close agreement of the mean value derived from elemental analysis with the human respiratory quotient (RQ) of 0.82 (see p 683, West and Todd, 1952). RQ is the ratio of the mols of CO_2 produced to mols of O_2 consumed in animal respiration. In a closed ecosystem, the AQ of the algal culture must match the animal RQ, or the system will go out of balance.

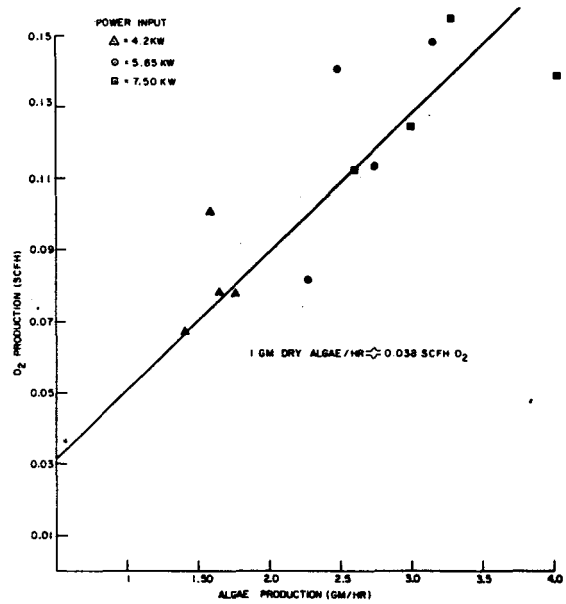


FIGURE 6 RELATIONSHIP OF O_2 PRODUCTION TO ALGAE PRODUCTION

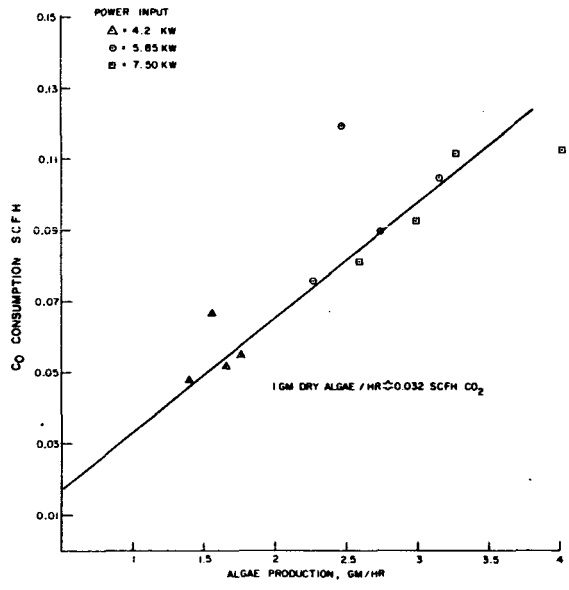


FIGURE 7 RELATIONSHIP OF CO_2 CONSUMPTION TO ALGAE PRODUCTION

Equilibrium Culture Density

The pattern of the analysis of variance on algal dry weight and packed cell volume were exactly the same as the pattern of O_2 production and CO_2 consumption. Power input significantly affected both measures of culture density, but agitation did not. Furthermore, no significant interactions were found.

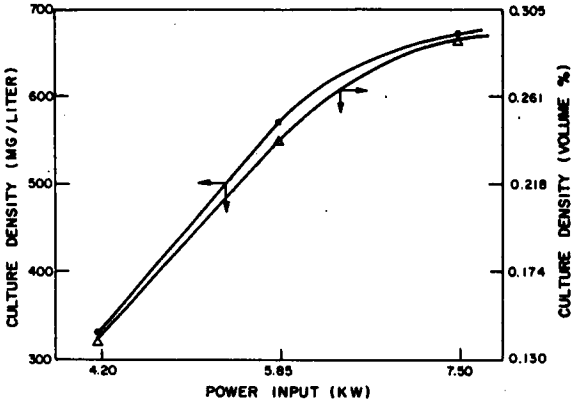


FIGURE 8 EFFECT OF POWER INPUT ON EQUILIBRIUM CULTURE DENSITY

The averages of the two measures of culture density were computed at each power input ignoring agitation rate. The average values for algal dry weight and packed cell volume, together with summary data, are presented in Table 4. The mean dry weights of the equilibrium algal suspensions at power inputs of 4.20, 5.85, and 7.50 KW were 326, 527, and 670 mg/L, respectively. Corresponding packed cell volumes were 0.14, 0.24, and 0.29% vol/vol. Plots of the data confirmed the curvature predicted by the components of variance.

Figure 8 shows that culture density increased with increasing power input. The increase in equilibrium culture density achieved by increasing power input from 4.20 to 5.85 KW was 246 mg/L or 0.10% packed cell volume. However, only a 98 mg/L or 0.05% packed cell volume increase was obtained by increasing power input from 5.85 to 7.50 KW. The data again indicate that a limit would soon be observed at higher power inputs with the lighting arrangement used.

Table 4

Effect of Power Input and Agitation on Culture Density

Power Input (KW)	4.20		5.85		7.50	
Agitation Rate (Cycles/min)	45	90	45	90	45	90
Dry Weight (mg/L)	296	326	445	594	618	805
	323	360	686	563	670	589
$\bar{X}$	309	343	566	578	644	697
$\bar{\bar{X}}$	326		572		670	
Packed Cell Volume (% vol/vol)	0.13	0.16	0.19	0.26	0.24	0.33
	0.11	0.15	0.30	0.24	0.28	0.30
$\bar{X}$	0.12	0.16	0.24	0.25	0.26	0.32
$\bar{\bar{X}}$	0.14		0.24		0.29	

In order to establish the relationship between the two measures of culture density, the data for dry weight and packed cell volume were plotted as shown in Figure 9. The regression line shows that 1 ml of wet algae or 1.0% (packed cell volume) is equivalent to 230 mg of dried algae with the methods used for these determinations. Thus, a conversion factor of 4.36 may be used in converting packed cell volume to dry weight. In other studies, described in Part II of this report, a conversion factor of 4.31 was found.

The derived values of algae production obtained by multiplying the harvest rate and algal suspension dry weight are presented in Table 5. Average values were 1.60, 2.66, and 3.22 gms/hr at power inputs of 4.20, 5.85, and 7.50 KW, respectively.

The calibrated nutrient metering pump, which governed the harvest rate of the algal suspension, was set to deliver nutrient solution at a rate of 4.9 L/hr. Periodic checks were made of pump delivery with the results shown in Figure 10. The figure shows that an algal suspension harvest rate of 4.8 L/hr was achieved by setting the nutrient feed rate and allowing the liquid level controls to discharge the algal suspension.

At a harvest rate of 4.9 L/hr and culture volume of 66L under steady-state conditions, approximately 1.8 times the total algae present in the PGE was harvested per day. Consequently, the time required for the culture to double in mass in steady-state culture was 9.3 hrs. In terms of specific growth rate, this corresponds to 2.6 $days^{-1}$ (2.6 doublings per day). The minimum doubling time reported for Chlorella 71105 is about 2.6 hrs, which corresponds to a specific growth rate of 9.2 $days^{-1}$ (9.2 doublings per day). The specific growth rate of 9.2 $days^{-1}$ however, was obtained under nearly idealized growth conditions (see Sorokin, 1959; also, Myers and Brown, 1961).

In studies reported in Part II of this report, a specific growth rate of 3.5 $days^{-1}$ was achieved under steady-state culture conditions. Therefore, it appears certain that the specific growth rate of 2.6 $days^{-1}$ in the pilot PGE can be surpassed.

The ash content of the algae is summarized in Table 6. A mean value of 5.5% was found when the algae were incinerated at 800°C. Parallel ash determinations in our laboratories resulted in a mean value of 6.3% (see Analytical Chemistry Section). The ash contents of the algae, in general, were variable, indicating a need for a more rigidly standardized preparative and analytical procedure, rather than any physiological change in the algae.

Elemental Composition of Chlorella

The analysis of variance of percentages of C, H, and N indicated that power input significantly affected the percentages of C and N but not

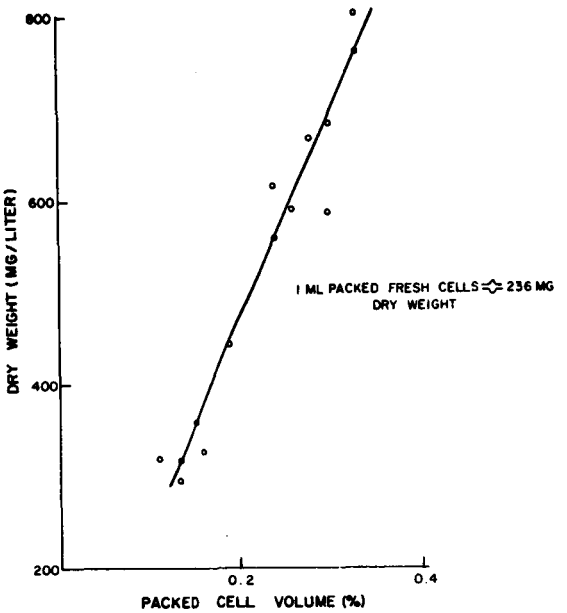


FIGURE 9 RELATIONSHIP OF DRY WEIGHT TO PACKED CELL VOLUME FOR CHLORELLA 71105

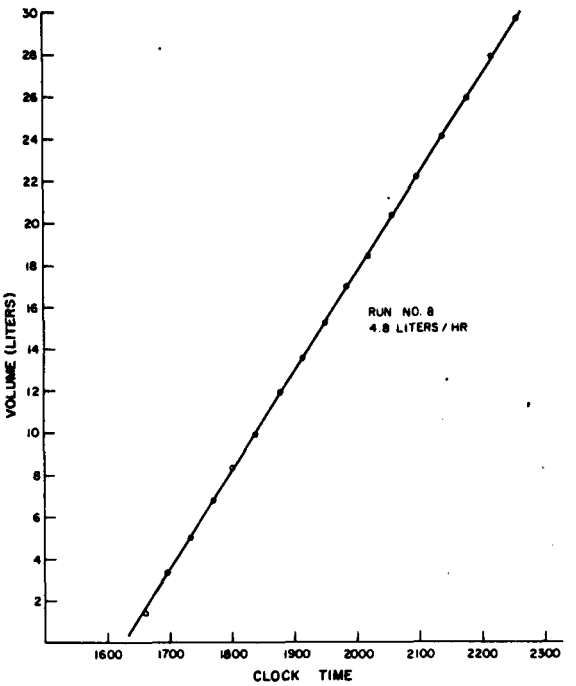


FIGURE 10 CUMULATIVE HARVEST OF ALGAL SUSPENSION

Table 5

Effect of Power Input and Agitation on Algae Production

Power Input (KW)	4.20		5.85		7.50	
Agitation Rates (Cycles/min)	45	90	45	90	45	90
Algae Production (gms/hr-dry weight)	1.42	1.66	2.26	2.75	2.60	4.02
	1.58	1.76	3.16	2.49	3.28	3.00
$\bar{X}$	2.71	2.62	1.50	1.71	2.94	3.51
$\bar{\bar{X}}$	1.60		2.66		3.22	

Table 6

Effect of Power Input and Agitation on Chlorophyll and Ash Content

Power Input (KW)	4.20		5.85		7.50	
Agitation Rate (Cycles/min)	45	90	45	90	45	90
Chlorophyll (%)	3.42	2.73	3.61	3.70	2.32	1.22
	4.25	3.48	---	3.85	3.66	2.45
$\bar{X}$	3.84	3.10	3.61	3.77	2.99	1.83
$\bar{\bar{X}}$	3.47		3.69		2.21	
Ash (%)	5.74	5.41	6.58	6.90	6.02	5.33
	5.72	3.26	2.72	6.31	7.16	5.18
$\bar{X}$	5.73	4.34	4.65	6.60	6.59	5.26
$\bar{\bar{X}}$	5.04		5.62		5.92	

Table 7

Effect of Power Input and Agitation on Carbon, Nitrogen, and Hydrogen Content

Power Input (KW)	4.20		5.85		7.50	
Agitation Rate (Cycles/min).	45	90	45	90	45	90
Carbon (%)	51.30	49.27	49.92	50.62	47.66	47.71
	50.72	52.08	53.46	50.64	50.52	48.14
$\bar{X}$	51.01	50.68	51.69	50.63	49.09	47.92
$\bar{\bar{X}}$	50.84		51.16		48.50	
Nitrogen (%)	10.79	9.38	10.80	10.90	9.74	9.95
	10.83	11.15	11.61	10.69	10.75	9.51
$\bar{X}$	10.81	10.26	11.20	10.80	10.24	9.73
$\bar{\bar{X}}$	10.54		11.00		9.98	
Hydrogen (%)	7.12	7.62	7.24	7.02	7.22	7.05
	7.08	7.11	7.34	7.06	7.22	7.10
$\bar{X}$	7.10	7.36	7.29	7.04	7.22	7.08
$\bar{\bar{X}}$	7.28		7.16		7.15	

of H. Agitation had no effect on the elemental composition of the algae, and no significant interations were observed within the limits tested. The major contribution to significant effects occurred at the highest power input. Mean values for percentages of C (Table 7) at power inputs of 4.20, 5.85, and 7.50 KW were 50.84, 51.16, and 48.50, respectively. Corresponding values for N (Table 7) were 10.54%, 11.00%, and 9.98%. An overall mean value of 7.20% H was obtained for the 12 samples of algae (Table 7). If two anomalous values are ignored, means of 11.2 and 9.8% N and 51.36 and 47.83% C are obtained at power inputs of 4.2 and 7.5 KW. The nitrogen and carbon content of algae are apparently influenced by light input.

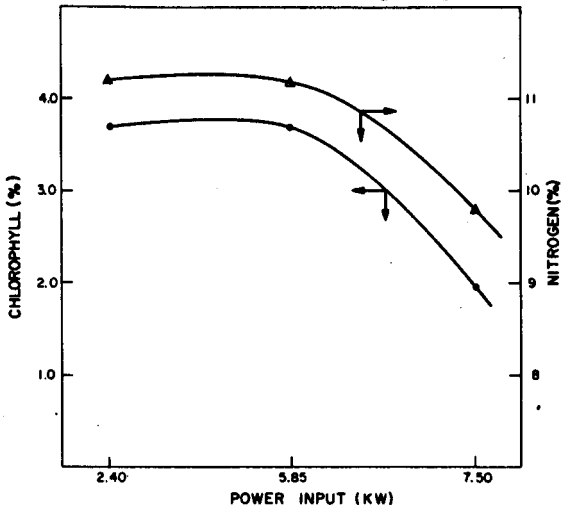


FIGURE II EFFECT OF POWER INPUT ON CHLOROPHYLL AND NITROGEN CONTENTS OF CHLORELLA 7110S

The average nitrogen values of the three power inputs are plotted in Figure 11. The decrease in N_2 content with power input is obvious.

From the elemental composition of algae, a number of derived values may be calculated: minimum molecular formula and weight; protein, carbohydrate, and lipid content; and a dimensionless R-value. The R-value gives an indication of the degree of reduction of the compound measured. In this case, of course, it would be the reduction value of all the substances constituting the alga. The derivation and detailed discussion of all derived values may be found in the Analytical Chemistry Section. A summary of average R-values and protein, carbohydrate, and lipid content at the three power inputs, exclusive of three anomalous values, are presented in Table 8. With increasing power input, there is a decrease in R-value and in protein and lipid, but an increase in carbohydrate content.

The average minimum molecular formulas and weights at different power inputs exclusive of these anomalous values are given in Table 9. The minimum molecular formulas ranged between $C_{5.3}$ $H_{9.2}$ $O_{2.1}$ N and $C_{5.7}$ $H_{10.3}$ $O_{2.6}$ N with corresponding molecular weights of 120.4 and 134.3.

Table 8

Effect of Power Input on Biochemical Composition of Algae

Power Input (KW)	R-Value	Protein (%)	Carbohydrate (%)	Lipid (%)
2.40	42.2	68.0	19.8	11.5
5.85	40.2	67.4	25.4	7.0
7.50	38.9	60.5	33.8	5.3

Table 9

Effect of Power Input on Minimum Molecular Formula

Power Input (KW)	Minimum Molecular Formula	Minimum Molecular Weight
2.40	$C_{5.5}H_{9.1}O_{2.1}N$	122.7
5.85	$C_{5.3}H_{9.2}O_{2.1}N$	120.4
7.50	$C_{5.7}H_{10.3}O_{2.6}N$	134.3

Chlorophyll and Ash Content of Chlorella

The means of duplicate determinations on chlorophyll and ash content of algae produced during the various experimental runs are given in Table 6. The results of variance analysis indicated that power input significantly affected algal chlorophyll content but not ash. Chlorophyll was lower at high power input. In Figure 11 we have drawn a smooth curve through the mean values for chlorophyll exclusive of three anomalous values at the three power inputs. The analysis of variance, however, indicated that the relationship did not depart significantly from linearity. Examination of the components of variance on power input revealed that the greatest decrease in chlorophyll content (1.47%) occurred by increasing power input from 5.85 to 7.50 KW. Agitation did not affect either dependent variable.

According to the summary data on chlorophyll in Table 6, the algae contained about 3.7% chlorophyll at a power input of 5.85 KW and 2.2% at a power input of 7.50 KW. This represents a 40% decrease in chlorophyll.

The decrease in chlorophyll content with light input has been observed by other workers. The important point of the observation, however, is that more oxygen was produced per unit chlorophyll, indicating a more efficient photosynthetic process at high light input. Calculation of the amount of O_2 generated per unit chlorophyll results in values of 27 and 45 L/hr/mg chlorophyll at light inputs of 5.85 and 7.50 KW.

A detailed tabulation of elemental analysis is given in Appendix D-3a. Sample calculations of the derivation of R-value; protein, carbohydrate, and lipid content; and minimum molecular formula and weight are given in Appendix D-5.

Operator Reliability

In order to determine if any fixed operator bias existed during the factorial experiments, the data collected by the various operators were also analyzed by the analysis of variance technique. No significant difference among the data of the operators was found.

Trace Gas Analysis

During experimental runs, 5-ml samples of gases from the exit stream were periodically subjected to analysis with Model No. 154 Perkin-Elmer vapor chromatograph. These analyses were run primarily to detect trace gases such as CO and CH_4 and also to check analytical values obtained with the infra-red CO_2 analyzer and paramagnetic O_2 analyzer.

The separation of major gases was accomplished by connecting I and J columns in series. The I column, as purchased, is packed with a synthetic zeolite and the J colums with silica gel, Type 15. The settings used for the gas chromatograph were as follows: pressure, 9.1 psig; temperature, 35°C; flow, 105 mm; voltage, 8 V; power, 35 W; sensitivity, 16 (O_2 and N_2) and 2 (CO_2).

Gas peaks were integrated by a Perkin-Elmer printing integrator and recorded on a Leeds-Northrup Speedomax recorder. Standard curves for O_2, CO_2, and N_2 were prepared from standard gas mixtures.

No evidence of any gas other than O_2, CO_2, and N_2 was found.

Material Balances

Material balances were calculated on the experimental runs in order to assess control of the process and reliability of the data. If the various substances in a chemical process can be completely accounted for, then it follows that the analyses for the process are reliable. Conversely, a poor material accounting reveals a process that is not functioning satisfactorily or that is not being controlled properly.

Three balances were calculated: a carbon balance, nitrogen balance, and a total gas balance. In each case, the balance consisted of comparing the total input to the total output of the material in question. Sample calculations are shown in Appendix C-4.

Table 10

Carbon and Nitrogen Balances

Run	Power Input (KW)	Agitation Rate (cycles/min)	Total Gas Accounting (%)	Carbon Accounting (%)	Nitrogen Accounting (%)
1	7.50	90	84.2	91.4	No Determination
3	7.50	45	66.2	86.0	"
4	7.50	90	74.6	96.6	"
5	4.20	90	72.8	97.3	101.1
6	5.85	45	109.5	102.3	109.3
8	4.20	45	91.1	94.2	106.2
9	7.50	45	86.4	97.9	No Determination
10	4.20	90	80.8	91.8	98.3
11	5.85	90	71.8	116.8	96.2
12	4.20	45	71.0	119.3	92.0
13	5.85	45	79.5	88.4	93.0
14	5.85	90	78.9	97.8	100.1

Carbon was supplied to the system in the form of carbon dioxide gas. The input was compared with the carbon leaving the system in the form of carbon dioxide gas and algae. The carbon content of the algae was determined by elemental analysis.

Nitrogen was introduced into the system in the form of urea in the nutrient medium. This input was compared to the nitrogen leaving the system as algae and unused urea. Although gaseous nitrogen was also supplied to the system, it is not utilized by the alga and therefore did not enter the balance calculations. The nitrogen content of the nutrient medium, the supernatant liquid, and algae were determined by the Kjeldahl procedure.

The total gas entering the system consisted of two inlet feeds of carbon dioxide and nitrogen. Both gases were introduced into the gas surge tank through calibrated rotameters. The combined input flows were compared with the vent gas flow which was measured by another calibrated rotameter in the vent line.

Gas accounting during the factorial experiments ranged from 66 to 110% with a median value of about 80%, as shown in Table 10. These accountings were considered reasonable since rotameters, with their inherent operator reading error, were used for measuring gas inputs and output.

Carbon accounting ranged from 86 to 119% with a median value of about 95%. The high carbon accountings indicate that Chlorella 71105 excretes very little organic material into the growth medium.

Nitrogen accountings were not made on Runs No. 1, 3, 4, and 9, but accountings on the remaining runs ranged from 92 to 109% with a median value of about 98%.

Light Measurements

Fundamental studies on photosynthesis have shown that photosynthetic rate is proportional to light intensity. The photosynthetic rate increases with light intensity until the photochemical apparatus is light saturated, a value characteristic of the plant. For maximum efficiency in the utilization of input light energy, the light energy supplied for photosynthesis must be some value less than the amount of energy required to maintain a maximum rate. Otherwise, the photochemical mechanism becomes light saturated, light energy is wasted, and the efficiency of the system is reduced.

For practical PGE design, in which lighting is but one factor, some compromise value of the ideal light intensity will no doubt be required. Although the approximate total energy requirement can be easily calculated, the most desirable distribution of the photosynthetically active energy within the algal suspension can only be determined empirically. The determination of the light intensities at various points in an algal suspension require technically difficult measurements. We investigated the problem in the manner that follows.

The description of light intensities within the algae suspension was divided into two parts: 1) determination of the light intensity at the culture surface and, 2) the determination of the light attenuation within the algal suspension. The intensity of the light transmitted to the culture surface is dependent on 1) lamp voltage, 2) thickness and number of interfaces of air, glass, and culture medium, 3) lamp age, and 4) the lighted surface area. The distribution of light within the algal suspension is a function of 1) surface light intensity, 2) culture depth, 3) culture density, and 4) the chlorophyll content of the cells.

Materials and Methods

To measure the intensity of the light transmitted to the culture chamber, the light contact chamber (consisting of the glass dome, lamp harness, and lamps) was removed from the system and positioned so as to simulate the actual operating conditions. The lamp voltages were adjusted to operating voltages of 200, 238, and 277V, and the light intensities were measured at selected positions about the periphery of the glass dome. For example, at a fixed height of three inches from the base of the dome, eight equidistant points were marked off around the circumference of the dome and the intensity at each point

was measured. This procedure was then repeated for other heights, e.g., six inches, nine inches, 12 inches, etc.

A Wollensak Fastax Exposure Meter, Model 755 (0-300,000 foot-candles range) was used to measure the light intensities. Measurements made on an optical bench, using a calibrated standard lamp (2350 candle power) and a precision voltmeter, indicated that the Fastax meter was reading within 1% of the indicated values on its basic range (0-3000 foot-candles). Since the other ranges (0-30,000 and 0-300,000) are obtained by addition of neutral density filters to the original meter, a conservative error of 5% was assigned to the instrument.

The distribution of light within the algal suspension was calculated based on the measurements of Myers and Graham, 1959. The actual measurements were obtained with an Ektron cell (lead sulfide) and calibrated neutral filters. The data presented were transformed to working equations. The derivation of these equations is described in Appendix C-5.

Table 11

Equations for Light Intensity at the Surface of the Glass Dome

The general expression for surface intensity was found to be:

$$I_o = a_o + a_1 y + a_2 y^2 + a_3 y^3 + a_4 y^4$$

where I_o = surface light intensity, foot-candles

y = vertical distance from base of dome, inches

The values for the coefficients are listed below:

	Lamp Voltage		
	200 V	238 V	277 V
a_0	10,713.48	18,731.70	33,568.21
a_1	- 5,053.64	- 8,790.70	-15,771.50
a_2	929.46	1,612.71	2,829.66
a_3	-52.66	- 91.48	- 159.96
a_4	0.92	1.61	2.82

Results and Discussion

The results of light measurements at the surface of the glass dome, in effect the algal suspension-glass interface, are presented in Figure 12. The light intensity normal to the surface of the dome was recorded for the three operating voltages (200, 238, and 277 volts). Each value given represents an average of eight measurements taken at equidistant points about the circumference of the dome. The maximum variation (2,000 foot-candles) in the eight measurements was observed at an operating voltage of 277V. Figure 12 shows the effect of lamp voltage on the resultant light intensities. The equations for these curves, as determined by the method of least squares, are given in Table 11. As anticipated, the highest intensity was obtained when the lamps were operated at the highest voltage. The maximum intensity (35,000 foot-candles) along the surface of the dome was at a point 15 inches above the base of the dome.

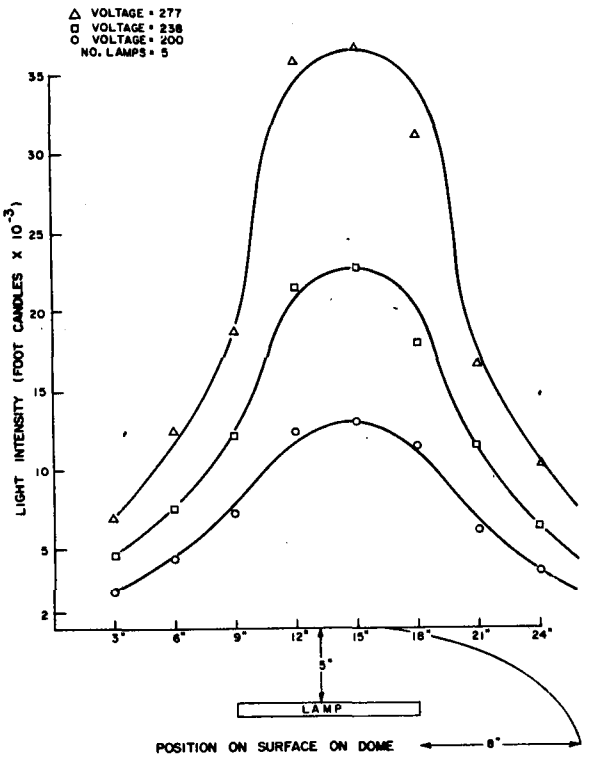


FIGURE 12 LIGHT INTENSITY AS A FUNCTION OF LOCATION ON DOME

The data of Myers and Graham on the light distribution within algal suspensions were analyzed by dividing the measurements according to the suspension depths and culture density.

Four equations were derived to describe four situations.

Situation I

$x > 75$ mm
$C_A < 230$ mg/liter

$$OD = 0.217\ C_A^{0.515} \log x - 0.561\ C_A^{0.409} + (10^{-5})\ x^2 \log C_A$$

Situation II

$x > 75$ mm
$C_A > 230$ mg/liter

$$OD = 1.43\ C_A^{0.168} \log x - 5.2$$

Situation III

$x < 75$ mm
$C_A < 230$ mg/liter

$$OD = \frac{x}{75} (0.00585\ C_A + 0.12)$$

Situation IV

$x < 75$ mm
$C_A > 230$ mg/liter

$$OD = \frac{x}{75} (0.00372\ C_A + 0.64)$$

where:

x = culture depth (mm)
C_A = culture density (mg/liter)
OD = optical density

For culture depths less than 75 mm (situations III and IV), the equations are similar to Beer's Law. However, plots of the equations do not go through the origin. (Beer's Law can be expressed as $OD = \Sigma C_A x$, where Σ is a constant.) This was anticipated because agreement with Beer's Law can be expected only when essentially monochromatic light is used, and in this case white light was being measured.

At culture depths greater than 75 mm, the linear relationship no longer exists.

The equations for surface light intensity (Table 11) were combined with the equations for light distribution in the culture, thus producing a general expression for the light intensity at any given point within the light contact chamber. The derivation of this expression is given in Appendix C-5. A graph of this general light intensity function is shown in Figure 13. The graph, based on a voltage of 277 volts, represents the maximized expression. As such it shows that the algae were exposed to intensities in the range 0-35,000. Therefore, cells very close to the surface of the glass dome at any given instant were exposed to light intensities well above saturation levels (about 2,000 foot-candles). However, the distribution of intensities indicates that the time of exposure to very high intensity light was rather short.

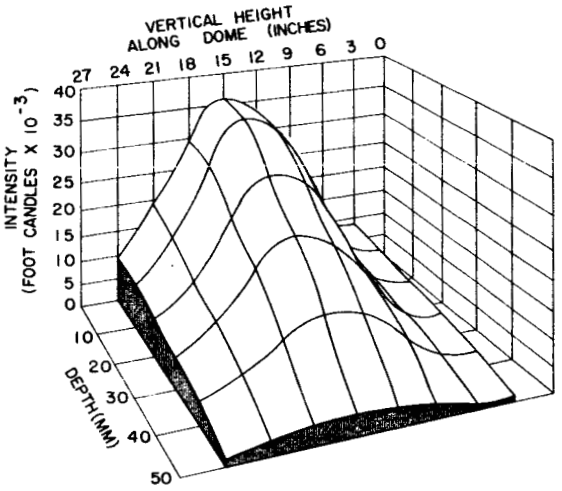


FIGURE 13 LIGHT INTENSITY DISTRIBUTION WITHIN THE PHOTOSYNTHETIC GAS EXCHANGER

It should be noted that the actual light intensities at various depths within the algal suspension may be slightly higher than calculated values. The light measurements of Myers and Graham were conducted with mildly aerated, stirred suspensions. The aerated culture in our pilot PGE occupied a volume of about 66 liters. Of this volume, approximately 1.4 liters were air bubbles. The culture depth correction for the transparency of air bubbles is consequently not more than 2%.

Turbulence within the Culture

States of motion or mixing in liquids are ordinarily described in terms of a dimensionless number, Reynolds number. This number has been defined for liquid flow through pipes and channels of simple cross-section and for liquids stirred in simple-shaped vessels. In order to apply the number to our PGE, it was necessary to make certain assumptions. Furthermore, it was concluded that this parameter for a flowing liquid could be more simply applied than the modulus for a stirred liquid, even though the latter is superficially more apt. The configuration of the vessel is shown in Figure 1 and in Figure 2, a cutaway view of the PGE. Figure 14 is a diagrammatic vertical section through the vessel with the rough dimensions used in the calculation of Reynolds number.

For a flowing liquid, the modulus is defined as follows:

$$Re = \frac{D_e V \rho}{\eta} \quad \text{where}$$

D_e is 4 times the ratio of the area of the channel to its wetted perimeter,

V is the velocity of flow,

ρ is the density of the liquid, and

η is its viscosity.

The channel was considered to have two independent halves, a lower rectangular section, and an upper triangular one. The velocity of "flow" in each half was taken to be average velocity of the agitation at the midpoint of the channel. During the operation of the PGE, the agitator was driven through a stroke of 16.5° at 45 or 90 cycles per minute. Reynolds numbers for the slower speed were calculated as follows:

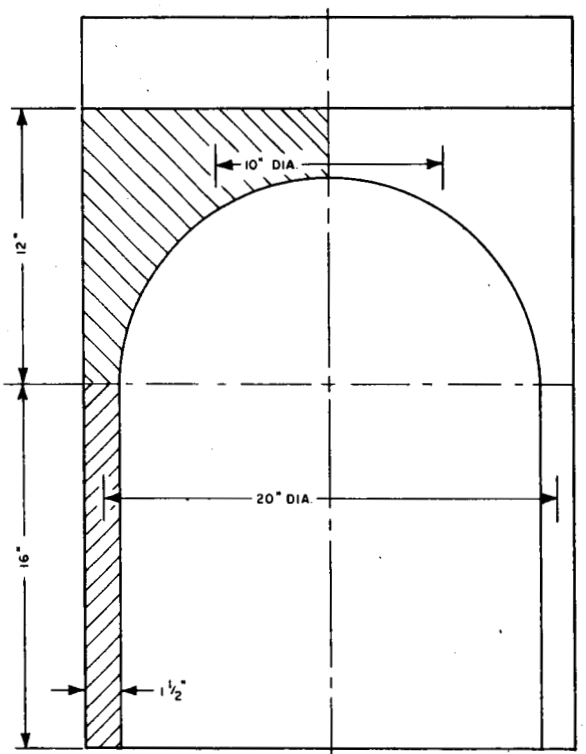


FIGURE 14 DIAGRAMMATIC VERTICAL SECTION ALGAL SUSPENSION CHAMBER

For the rectangular half,

$$D_e = 4 \frac{16 \times 1.5}{2x16+2x1.5} \quad \frac{1}{12} \text{ ft}$$

$$V = \frac{16.5x\pi x20x45x2}{360x12X60} \text{ ft/sec}$$

$$\rho = 62.4 \text{ lbs/ft}^3$$

$$\eta = 4.6 \times 10^{-4} \text{ lbs/sec ft}$$

$$Re = \frac{0.23x0.36x62.4}{4.6x10^{-4}} \cong 110,000$$

For the triangular half,

$$D_e = 4 \frac{12x10/2}{12+10+15.6} \quad \frac{1}{12} \text{ ft}$$

$$V = \frac{16.5x\pi x10x45.2}{360x12x60} \text{ ft/sec}$$

$$\rho = 62.4 \text{ lbs/ft}^3$$

$$\eta = 4.6 \times 10^{-4} \text{ lbs/sec ft}$$

$$Re = \frac{0.53x0.18x62.4}{4.6x10^{-4}} \cong 130,000$$

Mixing or flow is considered turbulent rather than laminar when the Reynolds number is larger than 2000. Turbulent systems have no stagnant areas, and they improve material transport. The significant effects of turbulence on the distribution of light to algal cells in the culture is discussed below.

In addition to spectral distribution and intensity, light intermittency has been shown to affect photosynthesis. A more efficient utilization of high intensity light has been reported where the light was supplied in short flashes separated by longer dark periods (Phillips and Myers, 1954). Electric Boat Division designs have attempted to create the flashing or intermittent pattern through turbulence in the culture.

Figure 15 shows how such intermittency may be accomplished in the turbulent algae culture between the light source and outer wall in the annular region of the algae suspension chamber (see Figure 14).

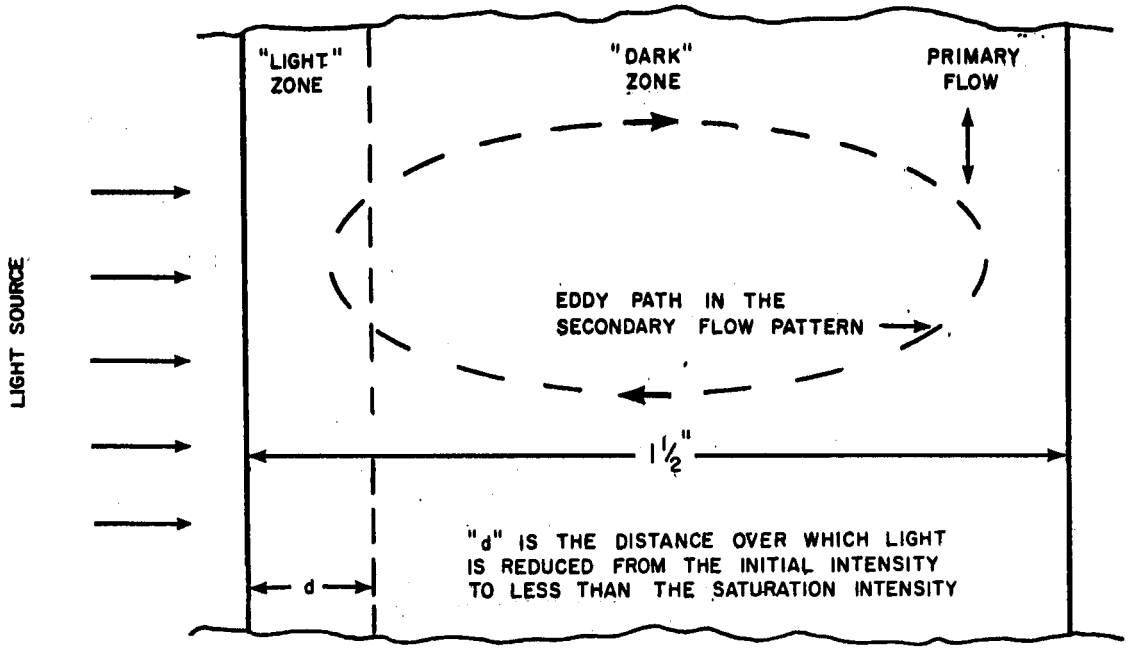


FIGURE 15 HYPOTHETICAL SECONDARY FLOW PATTERN IN A TURBULENT SYSTEM (HORIZONTAL PLANE)

In this system, if peripheral eddy speed is assumed constant, it is apparent that the desired light-to-dark ratio can be achieved with proper system geometry.

In the volume of solution perpendicular to the plane of Figure 15 represented by the distance, d, the degree of flashing, or intermittency, of the light is of a higher order than that resulting from the turbulent flow pattern. In this space, for example, a single algal cell (a space and time averaged cell, to be more precise) will "see" vast numbers of other cells on "looking" at the light source. Even relatively slow random movement of the myriad of cells(motion induced by thermal gradients, e.g.) would effect extremely rapid light shuttering upon the single cell. The rapidity of light shuttering caused by the passage of a large number of cells between the typical cell and the light source would be several orders of magnitude greater than that produced by secondary turbulent flow (the eddy path of Figure 15).

It is not necessary to derive a quantitative model of the flow pattern produced by the agitator. In descriptive terms, such flow may be

categorized as primary flow (the oscillatory circumferential movement resulting directly from agitator blade motion) and secondary flow. The most prominent secondary flow patterns result from fluid "leakage" past the agitator blades, between the light dome and outer wall, as indicated in Figure 16. In the experimental model, the agitator

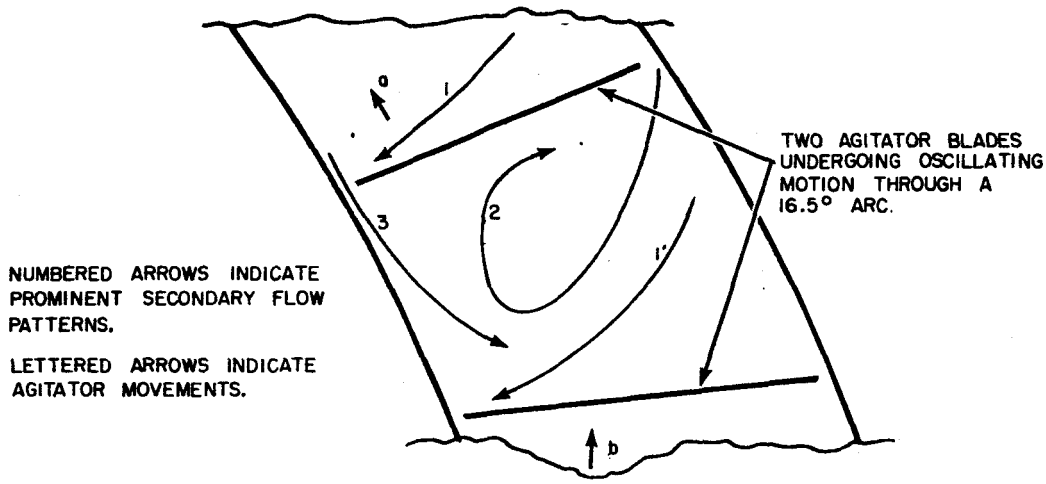


FIGURE 16 SECONDARY FLOW PATTERNS IN THE PGE (HORIZONTAL PLANE)

blades were designed with little pitch. However, the secondary flow patterns depicted in the illustration could be established even with blades of moderate pitch.

Other less significant secondary flow patterns are also generated in the system: motion imparted to the solution by rising gas bubbles*, motion resulting from thermal gradients near the lamp source, motion occurring at the top and bottom of the agitator blades, and interactions of these motions with each other and with the primary and predominant secondary flow patterns.

The question becomes: Did turbulence have any beneficial effects on light shuttering in the algal suspension chamber shown in Figure 14? Only definitive knowledge of the peripheral velocity of secondary flow eddies of types 1 and 2, Figure 16, would serve, in part, to solve the problem. Even then, more complex statistical information on eddy size

*Although turbulent flow can be imparted to a liquid by introducing gas at extremely high velocity (e.g., gas jets), bubbles rising by gravity through a liquid will not contribute appreciably to turbulence, since nearly all interfacial slip occurs in the gas bubble as it rises.

and rate of break-up would be essential before even an approximate answer could be obtained. Qualitatively, however, it seems reasonable to assume that no eddy velocity in the system would differ by more than an order of magnitude from the maximum linear (tip) velocity of the agitator blades.* Shear resistance of fluids in this viscous system would become extremely large, if higher velocities were encountered. In any event, it is safe to conclude that the average velocity of all small fluid elements is no greater than the agitator blade tip velocity.

In the present system, this blade tip velocity can be calculated: the rocker arm linkage to the agitator is such that the agitator rotates through 16.5° with the offset pulley set at 7/8-in. The maximum tip velocity of the six-in. rocker arm is equal to the tangential velocity of the offset pulley, which, at 90 cycles/min (the RPM of the offset pulley) is 495 in./min or 0.7 ft/sec. As the agitator blade tip diameter is 19 in., the maximum blade tip velocity can be calculated as 1 ft/sec. Therefore, the time required for a fluid element to traverse the average distance between "light" and "dark" zones in the 1-1/2 in. annular space is about 0.06 seconds. It should be noted that, in terms of the preceding analogy, the vast bulk of the algal cells would require traverse times far exceeding 0.06 seconds.

An algal cell exposed to light saturating intensity for about one millisecond requires about ten milliseconds to complete the sequence of steps in the portion of the photosynthetic reaction unaffected by light. (However, a saturated cell can continue to absorb light at the expense of its shaded neighbors.) Consequently, it is safe to conclude that light intermittency caused by agitation in the present experiment did not significantly benefit algal cell growth. It is also apparent, however, that an order of magnitude increase in impeller velocity or improved impeller design should result in more turbulence than that obtained in this experiment. It appears reasonable to expect eddies of the type shown in Figure 15 by designing for proper agitator blade angle and spacing of the blade edge from the light chamber and from the outside wall of the culture chamber. It is conceivable that with improved hydrodynamic design and no increase in agitator velocity, the period required for eddies to transport algal cells from "dark" to "light" zones and back would approach ten milliseconds.

The concept of an eddy period should be useful in future descriptions of light shuttering. A.G. Frederickson, in a personal communication, described cinematographic experiments in which enhancement of photosynthesis by light intermittency was demonstrated.

---

*Some elements of fluid could move faster than the agitator in certain situations. An element of fluid, for example, moving at blade velocity toward a blade that had reached the end of its stroke, stopped, and then started in the reverse direction, would move into the lower pressure region of the wake. To conserve momentum, the speed of the particle would increase. It is momentum, rather than velocity, which is conserved in this system.

SECTION IV

CHEMICAL ANALYSES

Page intentionally left blank

SECTION IV

CHEMICAL ANALYSES

INTRODUCTION

During the steady-state operation of the photosynthetic gas exchanger in the factorial series of runs, samples of the Chlorella culture were collected. Freeze-dried cells were prepared from these samples of the suspension and analyzed for moisture, ash, chlorophyll, and nitrogen. Nitrogen was also determined in samples of the supernatant from the centrifuge and in samples of nutrient medium from the storage tanks. (The medium contained a measured amount of urea, but the possibility of some hydrolysis and loss of nitrogen from stored medium had to be checked.)

Weighed subsamples of freeze-dried algae were sent to Galbraith Laboratories, Inc., Knoxville, Tennessee for analyses of carbon and hydrogen as well as moisture, ash, and nitrogen. These anlyses served as an independent check of work in our own laboratories.

The results of analyses were used for calculating material balances, R-values, minimum molecular formulae, assimilatory quotients, and biological compositions.

MATERIAL AND METHODS

To prepare Chlorella 71105 samples for analysis, 20-30 liters of the harvested suspension were centrifuged in an International Chemical centrifuge and the supernatant liquid was collected and refrigerated. The cells were then washed with deionized water and re-centrifuged three times. The washed algal paste was spread on an aluminum plate, frozen, and placed in a vacuum dryer at 120°F for eight hours. The dried material was pulverized to a fine powder with mortar and pestle, transferred to a glass jar, and stored in a refrigerator.

The prepared samples of dried algae were then analyzed.

Nitrogen. The AOAC (1955) Kjeldahl method was used. Aliquots of the stored medium and samples of supernatant liquid from the centrifuge were also analyzed for nitrogen content by this method.

Moisture. Samples of freeze-dried algae were weighed in aluminum dishes and placed in an oven overnight at 105°C. The loss of weight of the samples was ascribed to moisture.

Ash. Dried samples were incinerated in a muffle furnace for two hours at 650°C.

Chlorophyll. The freeze-dried sample was extracted with anhydrous ethyl ether. The optical density of the extract was determined in the Bausch and Lomb spectrophotometer according to the AOAC (1955) method. Details of the method for chlorophyll are given in Appendix D-1.

Other Analytical Determinations. In addition to the chemical analyses listed above, the following physical measurements were regularly made: 1) packed cell volume (PCV) of the culture suspension, 2) dry weight of residues from measured volumes of suspension, and 3) dry weight of measured volumes of washed cells. Detailed procedures are given in Appendix D-2. The pH of the stored medium and the centrifuge supernatant was measured with a Photovolt Model 115 pH meter.

RESULTS AND DISCUSSION

The results of analyses from the twelve runs in the factorial series and from two additional runs (2, 15) are given in Table 12. Complete analytical data for our laboratory and Galbraith's are presented in Appendix D-3a and 3b. Values for moisture found in our laboratory were consistently higher than those found by the outside laboratory, which merely reflects the difference in the drying temperatures used. Ash content as determined in our laboratory was consistently higher than the values determined by the outside laboratory. The difference here also is a reflection of the temperatures used. Agreement between the two laboratories in nitrogen determinations was very good; the consistently lower values from the outside laboratory reflect the difference in moisture values used to correct raw values to a moisture-free basis.

From inspection of the data in Table 12, moisture, ash, and nitrogen content were independent of light intensity (power input) and agitation rate. A possible effect of light intensity on nitrogen content is discussed in the paragraphs on chlorophyll analyses. Average values for our analyses of factorial runs were as follows:

Moisture	8.67%	
Ash	6.39%	(moisture-free basis)
Nitrogen	10.76%	(moisture-free basis)

The exceptionally low ash values for runs 13 and 10 are included in the average.

Chlorophyll Analyses

The results of chlorophyll analyses for the factorial runs are given in Table 13.

Table 12

Comparison of Analyses by
Electric Boat Division and an Independent Laboratory

Run	Power Input (KW)	Agitation Rate (cycles/min)	Moisture (%)		Ash (%)		Nitrogen (%)	
			EB[a]	Galbraith[b]	EB[c]	Galbraith[d]	EB	Galbraith
8	4.20	45	8.33	7.83	7.05	5.74	11.05	10.79
12	4.20	45	8.83	7.69	6.46	5.71	11.23	10.83
5	4.20	90	7.01	3.69*	5.98	5.41	9.69	9.37
10	4.20	90	7.10	7.43	4.02	3.26	11.32	11.15
6	5.85	45	10.46	7.87	7.25	6.57	10.85	10.79
13	5.85	45	8.77	8.31	3.18	2.72	11.72	11.61
14	5.85	90	9.15	7.94	7.93	6.90	10.97	10.90
11	5.85	90	8.76	7.46	7.02	6.31	11.33	10.69
3	7.50	45	8.51	4.47*	6.99	6.02	9.91	9.74
9	7.50	45	9.39	7.83	7.33	7.15	11.20	10.75
1	7.50	90	9.54	4.08*	6.45	5.33	10.01	9.95
4	7.50	90	8.24	4.50*	6.99	5.18	9.89	9.51
$\bar{X}$			8.67		6.39		10.76	
2	7.50	0	9.35	4.10*	6.69	6.49	10.00	9.32
15+	7.50	60	7.56	7.52	4.53	3.92	9.83	9.36

a Dried at 105°C

b Dried at 100°C except for the (*) values; these samples were dried at 40°C under reduced pressure

c Incinerated at 650°C

d Incinerated at 800°C

\+ 5% CO_2 was used in this run

Total chlorophyll in dried algae samples varied from 1.22 to 4.25%. This is in reasonable agreement with recent literature. Myers and Graham (1959) reported 1.7 to 4.4%; Sauberlich (personal communication) reported 2.58% chlorophyll in mass-cultured Chlorella. A higher range (4-6%) was reported in the 1953 Carnegie Report (Burlew, 1953).

From Table 13 it is obvious that agitation rate had no effect on the chlorophyll content. There was, furthermore, no significant difference in the chlorophyll content of cultures grown at 4.20 or 5.85 KW. At 7.50 KW, chlorophyll was significantly decreased. The average chlorophyll values were 3.4%, 3.7%, and 2.4% at 4.20, 5.85, and 7.50 KW respectively.

Table 13

Effect of Power Input on Chlorophyll Content and Equilibrium Density

Run	Power Input (KW)	Agitation Rate (cycles/min)	Chlorophyll (%)	Suspension Density	
				Packed Cell Volume (%)	Gravimetric Density (mg/l)
8	4.20	45	3.43	0.13	296
12	4.20	45	4.25	0.11	322
10	4.20	90	3.48	0.15	360
5	4.20	90	2.73	0.16	326
6	5.85	45	3.61	0.19	445
13	5.85	45	2.45*	0.30	686
14	5.85	90	3.70	0.26	594
11	5.85	90	3.85	0.24	563
3	7.50	45	2.32	0.24	618
9	7.50	45	3.66	0.28	670
4	7.50	90	2.45	0.30	589
1	7.50	90	1.22	0.33	805

*Sample 13 was overheated during drying.

Table 13 also shows the effect of power input on equilibrium suspension density. As discussed in detail in the section on the factorial experiment, the suspension density increased significantly with increasing light intensity. Both effects have been previously reported by Myers and Graham (1959).

The effect of light on chlorophyll content is more obvious if some anomalous values are noted, as in Table 14. This table isolates anomalous values and gives the nitrogen values for the factorial series. Run 5, which has a low chlorophyll value at 4.20 KW, also showed low nitrogen. The sample for Run 9, which was inordinately high in chlorophyll, was likewise high in nitrogen. With these runs eliminated, the effect of high light intensity on chlorophyll becomes more obvious, and the possibility that nitrogen was significantly low at 7.50 KW becomes apparent.

Chlorophyll-a contents for samples from the factorial series were calculated by the AOAC method. The values ranged from 58 to 64% of the total chlorophyll.

Table 14

Effect of Power Input
on Nitrogen and Chlorophyll Content

Run	Power Input (KW)	Chlorophyll (%)		Nitrogen (%)	
8	4.20	3.43		11.05	
12	4.20	4.25	3.72	11.23	11.20
10	4.20	3.48		11.67	
5	4.20	2.73		9.69	
6	5.85	3.61		10.85	
13	5.85	--*		11.72	
14	5.85	3.70	3.72	10.97	11.21
11	5.85	3.85		11.33	
3	7.50	2.32		9.91	
1	7.50	1.22	1.99	10.01	9.85
4	7.50	2.45		9.89	
9	7.50	3.66		11.20	

*Sample 13 was overheated during drying; it was brown rather than green in color.

A statistical evaluation of 23 random values of the pH of the algal suspension indicated that the pH was constant throughout the factorial experiment. The pH of the suspension was found to be 5.2 ± 0.1.

Nitrogen Balance

One way of checking control of continuous processes and the accuracy of analyses and measurements is to compute material balances or accountings for the process. The nitrogen balance compares the amount of nitrogen entering the system with the amount leaving. In the operation of the gas exchanger, the sole source of nitrogen entering was urea in the nutrient medium. (Nitrogen gas was also fed into the system, but there is no reason to suspect N_2-fixation.) Nitrogen left the system in two parts: 1) in the cells of the harvested suspension, and 2) in the supernatant from centrifuging the harvested suspension.

Table 15 gives nitrogen balance data for eight runs of the factorial series. A sample calculation is given in Appendix C-4. Nitrogen balance in the eight runs varied from 92 to 109%, indicating fair process control and analyses.

Table 15

Nitrogen Balance in the Factorial Series

Run	I N in Nutrient Medium mg/liter (by analysis)	II N in Supernatant mg/liter (by analysis)	N Consumed mg/liter (I-II)	III N in Dried Cells mg/liter (by analysis)	N Balance % Recovered ($\frac{II+III}{I} \times 100$)
8	101.6	75.2	26.4	32.8	106
12	104.9	60.3	44.6	36.2	92
5	97.1	66.6	30.5	31.6	101
10	100.8	58.4	42.4	40.8	98
6	94.9	55.5	39.3	48.3	109
13	98.2	10.9	87.2	80.4	93
14	72.6	10.9	61.7	61.8	100
11	116.6	43.6	73.0	67.5	96

Minimum Formula Weight

Table 16 gives the elemental composition of samples from the factorial series. From these data, minimum molecular weights and approximate empirical formulae for "algal protoplasm" were calculated according to a method given by Myers (1957). The molecular formulae are also given in Table 16. The method for calculating formulae is given in Appendix D-4. The average empirical formula for Chlorella was $C_{5.56}\ H_{9.62}\ O_{2.26}\ N$; the corresponding molecular weight is 132.0 if the average ash content (5.52%) is taken into account. Myers (1957) found $C_6\ H_{11.1}\ O_{2.7}\ N$ and a molecular weight of 149, including ash.

Assimilatory Quotient

The assimilatory quotient (AQ) in photosynthesis is defined as the ratio of the number of moles of carbon dioxide absorbed to the number of moles of oxygen produced: CO_2/O_2. This parameter can be obtained from gas analyses, and it can be estimated from the synoptic equation for photosynthesis with data on the elemental composition of the algae used. The method is given in Appendix D-4. The calculated AQ values for the factorial series are given in Table 16. As seen in the table, the AQ calculated from chemical analyses varied but little, from 0.82 to 0.85, with an average value of 0.83. Myers (1957) reported an average value of 0.82 for urea-grown Chlorella. Both values are very close to the theoretical AQ for urea-grown cells.

Table 16

Elemental Analyses of *Chlorella* 71105

Run	Power Input (KW)	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Ash (%)	AQ	Molecular Formula C	H	O	N
8	4.20	51.29	7.12	10.79	5.74	.83	5.5	9.3	2.0	1
12	4.20	50.71	7.09	10.83	5.71	.83	5.5	9.2	2.1	1
10	4.20	52.08	7.11	11.15	3.26	.84	5.5	8.9	2.1	1
5	4.20	49.21	7.61	9.37	5.41	.82	6.1	11.4	2.7	1
6	5.85	47.54	7.23	10.79	6.57	.83	5.1	9.4	2.3	1
13	5.85	53.46	7.33	11.61	2.72	.83	5.4	8.8	1.9	1
14	5.85	50.63	7.02	10.91	6.90	.83	5.4	9.0	2.0	1
11	5.85	50.39	7.05	10.69	6.31	.83	5.5	9.2	2.1	1
9	7.50	50.52	7.23	10.75	6.02	.82	5.5	9.4	2.1	1
3	7.50	47.66	7.23	9.75	7.15	.83	5.7	10.4	2.5	1
1	7.50	47.71	7.05	9.95	5.33	.85	5.6	9.9	2.6	1
4	7.50	48.14	7.10	9.51	5.18	.85	5.9	10.5	2.8	1

Reduction Value

Spoehr and Milner (1949) introduced the concept of reduction value (R-value). According to this concept, the oxygen-absorbing power of cells produced in photosynthesis is strictly related to the chemical composition, and this power can be estimated from the AQ of photosynthesis or from the elemental composition of cells produced in the reaction. A sample calculation is found in Appendix D-5. The formula for calculating R-value was given by Spoehr and Milner as:

$$R = \frac{\%C \times 2.664 + \%H \times 7.936 - \%O_2}{3.99}$$

According to this formula, R_{CO_2} is zero, and R_{CH_4} is 100. R-values for carbohydrate, a typical protein, and a typical lipid are 26, 42, and 67, respectively. Spoehr and Milner reported R-values for *Chlorella* between 38 and 63. Table 17 gives R-values for *Chlorella* 71105 calculated from elemental analyses. The average value for the factorial runs was 40.8. As seen from this table, the R-value varied very little, but tended to fall with increasing light intensity while the oxygen content of the minimum molecular formula increased as seen in Table 16. Since the oxygen content is found by difference, it is a reflection of the effect on carbon and nitrogen. This trend is more evident if the anomalous values for runs 9 and 13 are excluded as discussed with regard to chlorophyll and nitrogen content.

Table 17

Reduction Values and Calculated Proximate Composition of Chlorella 71105

Run	Power Input (KW)	R Value	Protein (%)	Carbohydrate (%)	Lipid (%)
8	4.20	42.5	67.4	19.9	12.7
12	4.20	41.7	67.7	21.5	10.8
10	4.20	42.3	69.0	18.0	11.0
5	4.20	41.0	58.0	29.2	12.2
6	5.85	39.3	67.4	27.7	4.8
13	5.85	43.3	72.6	14.3	13.2
14	5.85	39.9	68.1	25.9	6.0
11	5.85	41.4	66.8	22.8	10.3
9	7.50	42.1	67.1	21.0	11.8
3	7.50	39.1	60.0	35.3	3.8
1	7.50	38.7	62.2	32.8	4.9
4	7.50	39.2	59.4	33.3	7.3
$\bar{X}$			65.4	25.1	9.0

Biochemical Composition

The protein content of plant tissue can be estimated closely from nitrogen analyses. A figure of 6.25 times the nitrogen content has been widely used. Knowing the protein content of a sample of plant tissue or algae and the R-value, the carbohydrate and lipid contents can be calculated by setting the known R-value equal to a weighted sum for carbohydrate, lipid, and protein:

$$\text{Protein}(\%) \times 42 + \text{Carbohydrate}(\%) \times 28 + \text{Lipid}(\%) \times 67 = 100\% \times \text{R-value} \quad (1)$$

$$\text{Protein} + \text{Carbohydrate} + \text{Lipid} = 100\% \quad (2)$$

A sample calculation is given in Appendix D-5.

Table 17 gives the proximate biochemical composition of samples from the factorial series. The average estimated composition for Chlorella 71105 was 65.4% protein, 25.1% carbohydrate and 9.0% lipid. The slightly decreased protein content at high light intensity is a direct consequence of the nitrogen values already discussed. The increase in carbohydrate at the expense of lipid at high light intensity is also worthy of note. A comparison of biochemical composition with other

investigators is discussed in the section on the factorial experiments, as well as the relationship between light intensity and algal growth and photosynthesis.

SUMMARY

Fourteen freeze-dried Chlorella 71105 samples from a factorial series of runs in the photosynthetic gas exchanger were analyzed for moisture, ash, nitrogen, carbon, hydrogen, and chlorophyll. The results of these analyses and calculations derived from them can be summarized as follows:

1. The moisture and ash contents of the algae were independent of the light intensity and rate of agitation for the system.

2. The chlorophyll content of the algal samples was 3.4 and 3.7% when the lamp wattage increased from 4.20 to 5.85 KW. It decreased to 2.4% at 7.50 KW.

3. From 92-109% of the nitrogen was accountable in the nitrogen balance.

4. The average empirical minimum molecular formula was $C_{5.6}H_{9.62}O_{2.26}N$ and the molecular weight was 132.0 including ash.

5. The Assimilatory Quotient was 0.83.

6. The average R-value was 40.8 and it was observed to decrease with increased light intensity. This was due to the effect of light intensity on elemental composition.

7. The average proximate composition estimated from R-value and elemental analysis was protein 65.4%, carbohydrate 25.1%, and lipid 9.0%.

Page intentionally left blank

SECTION V

DISCUSSION

Page intentionally left blank

SECTION V

DISCUSSION

FACTORIAL EXPERIMENTS

In experimental work conducted in both Phases I and II, PGE performance with Chlorella 71105 was evaluated in steady-state or continuous cultures. This was done for a number of specific reasons.

Algae are subject to the same laws of population dynamics as other microorganisms. They exhibit a growth cycle, and their physiological activity changes during this cycle. Gas exchange measurements taken during "batch" growth reflect the mean physiological activity of the population during one transient phase of the growth cycle. Depending on the phase selected, gas exchange performance could be over- or under-evaluated. On the other hand, gas exchange measurements on algal cultures under steady-state growth conditions reflect the physiological activity of a mixed population of cells in all phases of the growth cycle. Theoretically, the rate of growth and gas exchange under steady-state conditions could be maintained indefinitely.

In fermentation technology, it has been demonstrated that when the greatest yield in cellular mass is desired, continuous processes are by far the most effective. Since O_2 production is a function of algae production, it follows that steady-state culture is the most desirable method.

The highest yield of algae obtained during Phase I and II experiments was 3.47 gm/liter/day. This yield was attained under steady-state culture conditions in a bench scale unit having a culture volume of 3 liters. To the best of our knowledge, this yield has been exceeded by only one investigator: Professor Myers of the University of Texas. Myers (1958) obtained a yield of 6.08 gm/liter/day with a culture of Anacystis nidulans in a unit having a culture volume of 337 ml.

The highest yield obtained with the pilot scale unit in Phase I was 1.06 gms/liter/day. The Phase I unit, fabricated from plastic, could not be used for another year's experimentation because of general wear-and-tear. As a consequence, a second unit was designed and fabricated for Phase II studies. In studies completed with this unit, a yield of 1.16 gms of algae/liter/day was obtained. The measured O_2 production was equivalent to the needs of about 0.15 man. Optimization studies with the Phase II unit have not yet been completed, and the yield of 1.16 gms/liter/day is not considered the maximum obtainable. It is anticipated that the performance of the PGE will be increased considerably by changing the lighting arrangement and increasing light input, CO_2 concentration, and gas stream recirculation rate.

Lighting

The lighting in the Phase II pilot-scale PGE consisted of five 1500-W incandescent lamps mounted vertically within the glass dome. This arrangement resulted in good light distribution on the sides of the PGE but less so at the top. Furthermore, operation of the lamps in a vertical position resulted in some lamp failures and reduced light output. (In future work, a new lamp harness will allow the use of two or more pentagonal tiers of lamps mounted horizontally within the dome. Such an arrangement will permit the use of higher energy input at lower intensity as well as at high intensity.)

Agitation

Agitation was not found to influence performance significantly. This was the case even though a minimal gas stream recirculation rate was selected in order to minimize the contribution of the gas stream to agitation; it was anticipated that PGE performance would be increased at higher mixing rates. Basic studies by other workers have shown that algae can integrate light inputs greater than the amount required for saturating the photosynthetic mechanism (See Philips and Myers, 1954 and Burlew, 1953). However, complete integration occurs only with a suitable light-dark intermittency ratio. Agitation also aids mass transfer. In most commercial aerobic fermentations, agitation as well as aeration is provided for mass transfer of gases and nutrients. Apparently, agitation did not aid either light utilization or mass transfer within the limits tested. In any case, the limits will be extended in future work to determine if this is, in fact, the case.

Assimilatory Quotient

The mean assimilatory quotient (AQ) for the algae grown on urea was found by elemental analyses to be 0.83. This AQ is close to the human respiratory quotient (RQ) of 0.82. Assimilatory quotients calculated on the basis of gas exchange measurements were more variable, ranging from 0.68 to 0.96. However, this was taken more as a reflection of the problems of precisely measuring low gas flows rather than actual changes in algal AQ. The constancy of the algal AQ based on elemental composition substantiates this interpretation.

For long-term operation in a closed gas system, good control of the AQ must be attained to maintain a fixed gas composition. This can be done by manipulating the nitrogen source fed to the alga and controlling factors known to affect algal biochemical composition. The results of analytical studies on algae provided in the PGE suggest that light input, for example, will affect biochemical composition. No evidence of an effect on AQ, however, was established.

ELEMENTAL ANALYSES

Light input was found to have a significant effect on the chlorophyll, nitrogen, and carbon content of the algae produced.

Chlorophyll content was reduced from 3.7 to 2.0% by increasing light input from 5.85 to 7.50 KW. Yet, algae production increased from 2.66 to 3.22 gms/hr. This means that more O_2 was produced per unit of chlorophyll and indicated that the photosynthetic reaction was more efficient. In effect, 40% less catalyst was producing approximately 15% more O_2. It appears, therefore, that cells containing a low chlorophyll content are more desirable for photosynthetic gas exchangers. The reduction in chlorophyll in algae grown at high light intensity has been observed by other workers (Myers, and Graham, 1959, for example). Algae apparently adjust their chlorophyll content to the available light present. The evaluation of any practical application of this phenomenon requires the determination of light utilization efficiencies of algae having high and low chlorophyll contents.

The percentage of nitrogen and carbon present in the algae was also decreased by increasing light inputs from 5.85 to 7.50 KW. However, the percentage decrease was much smaller than that obtained for chlorophyll. Both nitrogen and carbon content were reduced about 10%. Hydrogen content remained relatively constant. The results of changes in elemental composition is reflected in derived values such as R-value (reduction value), protein, carbohydrate, and fat content calculated from the elemental analysis. R-value, protein content, and lipid content of the algal cells decreased at the highest power input, 7.5 KW, whereas carbohydrate content increased. A protein value of 72% was obtained at the lower power input of 2.4 KW and 62% at the highest power input (7.5 KW). These protein values agree with the value of 65% reported by Lubitz (1961) for Chlorella (see Table 18) grown under fluorescent illumination.

The carbohydrate content of the algae increased from 17 to 30% at power inputs of 2.4 and 7.5 KW while the lipid content decreased from 11 to 5%. These values for carbohydrate and lipid are also in reasonable agreement with those obtained by Lubitz.

Table 18

Protein, Carbohydrate, and Lipid Content of Chlorella 71105*

	This Study	Lubitz (1961)
Protein %	62-72	65
Carbohydrate %	17-30	21
Lipid %	5-11	8

*Analyses are reported on a moisture-free and ash-free basis.

Ash and moisture content of the algae samples were variable. This was mainly due to analytical methodology. It is apparent that for comparisons within and between laboratories, a precise analytical procedure must be followed as well as rigorously standardized washing, harvesting, and drying procedures.

MATERIAL BALANCES

To evaluate system control and data reliability, carbon, nitrogen, and gas accountings were made during experimental runs with the PGE. The accountings, in general, indicated that the system was properly controlled.

The gas and carbon accountings relied on gas flow rates as measured by rotameters which resulted in a reproducibility of about 20%. Although a considerable range in accountings was observed among the various runs, median accountings of 80 and 97% were obtained for total gas and carbon, respectively.

The median gas accounting of 80% appears somewhat low. This could be due to some fixed calibration error. Although each of the three gas rotameters used in the system was calibrated in series with standard rotameters, some fixed error may have been introduced in calibrating the rotameters measuring N_2 input or total gas output. The basis of this supposition is the fact that total gas accountings were consistently low while carbon accountings, which involved a rotameter measuring CO_2 input, fluctuated about a mean value of 100%. The possibility of a consistent leak of the same order of magnitude is quite remote in view of the fact that the system was carefully and regularly surveyed for leaks with sensitive and reliable techniques.

The 97% carbon accounting agrees well with a 95% accounting obtained by Myers and Johnston, (1949). The high accounting is evidence that Chlorella 71105 does not excrete large quantities of organic compounds into the medium; otherwise, the carbon accounting would have been much lower. There is no question, however, that some organic compounds are excreted; a medium in which the algae have been grown for a long time shows a yellowish color. The color may, of course, be a bacterial product; we have never observed long-term pure cultures.

Nitrogen accountings were obtained from chemical analyses of the algae produced, the harvested supernatant liquid, and liquid feed rate through the system. The accountings obtained fluctuated by about 10% around a median of 100%. One source of variability in N_2 accountings resides in the delivery rate of the nutrient feed pump. Measured feed rates ranged from 4.8 to 5.0 liters per hour. The median N_2 accounting of 100% agrees well with the value of 95% obtained by Myers and Johnston and provides further evidence that only minor amounts of organic compounds were excreted into the medium by Chlorella.

GENERAL DISCUSSION

In 1956, the feasibility of photosynthetic gas exchange was discussed at a symposium sponsored by the Office of Naval Research. Various prominent scientists in the field of photosynthesis provided engineering estimates of PGE requirements. Some of the estimates given have

been essentially substantiated by our pilot plant data even though the PGE optimization program has not been completed. The estimates with respect to the amount of algae required, liquid volume of the algal culture, total volume of the system, O_2 production, and power requirements on a one-man basis, along with results obtained with the present unit, are presented in Table 19.

The table shows that Professor Myers' estimates were by far the most conservative and Dr. Burk's the most optimistic. The differences are mainly due to varying estimates on steady-state culture densities achievable and the growth rate of an alga at the high density.

Table 19

Estimates of Requirements for a One-man PGE*

Estimator	Wet Algae (Kg)	Culture Volume (liters)	Total Volume (ft^3)	Power (hp)	O_2/hr/kg Algae (liters)
Myers	2.3	230	80	10	11
Bassham	1.0	100	20	6	25
Burk	0.25	25	--	1	150
This study	1.2	400	140	72	20

*Taken from ONR Symposium Report ACR-13, 1956.

Our data show a requirement of 1.2 Kg of wet algae for the support of one man. In other words, 1.2 Kg of algae must be maintained in a PGE under steady-state growth conditions. The value obtained with the pilot PGE (1.2 Kg) approaches the estimate of 1.0 Kg given by Bassham. Myers and Burk estimated requirements of 2.3 and 0.25 Kg, respectively.

The algal suspension volume requirement of 400 liters shown by the pilot PGE data is higher than the 25, 100, and 230 liter estimates made in 1956. This is due to the 0.29% culture densities obtained in the present pilot PGE as contrasted to symposium estimates based on 1% culture densities. The eventual culture volume will be determined by a trade-off of PGE weight and volume, culture density, growth rate, lamp volume, and power requirements. The chances of reducing the culture volume requirement by a factor of 2 to 4 appear very good. Steady-state culture densities of 1% have been maintained in bench-scale growth units in our laboratories for long periods. The reduction in culture volume will result from changes in configuration and lighting arrangement in the pilot unit.

The 20 liters of O_2 generated/Kg algae/hr found with the pilot PGE approaches the 25-liter estimate of Bassham. This value (specific yield) is a function of the growth rate of the alga used and the extent to which growth requirements of the alga are met. It will be difficult to increase the specific yield by any order of magnitude in the pilot PGE unless a new organism is found with a higher intrinsic growth rate than Chlorella 71105. Better design will allow higher culture densities, but it is doubtful that the growth rate can be increased because the two

maxima are in opposition. A blue-green alga has been reported that is capable of growing at a higher temperature than Chlorella 71105 (Gafford and Dyer, 1961); however, it apparently does not grow any faster.

No attempt was made to minimize power requirements in the pilot-plant PGE. High intensity lamps were used because of their compactness and the desirability of simulating sunlight intensities present in space. If illumination is provided artificially, a large power requirement is predicted although certainly not the requirement predicted by our present PGE. The actual requirement will very likely range about the value given by Bassham. The large power requirement is due to the low efficiencies in converting electricity to light and the manner in which visible light is utilized by presently known algae. The development of more efficient types of lamps or the discovery of algae capable of utilizing more light per unit time would, of course, alter this prediction.

The light energy to algae conversion efficiencies obtained with the experimental pilot PGE, calculated on the basis of O_2 production and algae production are presented in Table 20. The calculations show that a light energy conversion efficiency of about 3-5% was obtained with the incandescent lighting and lighting arrangement used in the PGE. An efficiency of 22% was reported by Kok (1952). In nature, efficiencies of about 5% are commonly observed in the conversion of sunlight energy to plant matter (See Burlew, 1953, and Tamiya, 1957).

Table 20

Light Conversion Efficiencies

Total Energy Input (KW)	(Cal/day)	Light Energy Input (Cal/day)	Energy Yield* O_2 (Cal/day)	Algae	Efficiency O_2 (%)	Algae
4.20	8.7×10^7	4.8×10^6	2.92×10^5	2.04×10^5	4.4	4.2
5.85	12.1×10^7	9.3×10^6	4.33×10^5	3.39×10^5	4.6	3.7
7.50	15.5×10^7	15.5×10^6	4.76×10^5	4.10×10^5	3.1	2.6

*Basis: 5.31 cal/cc O_2; 5.32 Kcal/gm algae

As mentioned earlier, the decision on type of lighting will involve trading off the various parameters of lighting such as bulk, weight, and power requirements. Further insight into the energy conversion efficiencies attainable will be obtained in additional experiments. Also important in the future program will be increased turbulence, which hopefully can increase efficiency.

The total volume for a one-man PGE, according to pilot PGE data, would require 140 ft^3 as compared to symposium estimates of 20 and 80 ft^3. No attempt was made to restrict the total volume of the system in the

experimental units. With further experimentation, the probability is good that this total volume requirement can be reduced by a factor of 2 to 4.

We have not only confirmed some of the predictions of the symposium participants but may well surpass them. During the past two years, we have accumulated a considerable amount of information concerning characteristics of the alga, Chlorella 71105, its management in mass culture, and PGE design. Time has not permitted the translation of all experimental information into an optimal PGE design; in fact, we have proposed one additional year's experimentation to gain further design information.

For example, with respect to the characteristics of Chlorella 71105, we have found that the organism can be continuously cultured over long periods of time without apparent change in growth characteristics. Furthermore, suspension densities up to 1% by volume have been obtained in continuous culture.

The organism will tolerate a remarkable range of environments. For example, we have noted that the organism is not harmed by temperatures ranging between 0° and 42°C, CO_2 partial pressures ranging between 0.03% and 10%, light intensities ranging from 0 to 40,000 foot-candles, pumping rates in half-inch pipe ranging from 0 to 10 gpm, pH ranging from 4 to 8, and total salt concentration in the medium ranging from 0.05% to about 1%.

We have observed neither excretion of inhibitory substances nor stimulation in growth by additives with Chlorella 71105. The organism can be grown in the same batch of medium for at least 71 days. Introduction of additives such as soil extracts to the medium does stimulate growth. However, substances leached from some types of rubber or metallic alloys can be highly toxic. Streptomycin and other antibiotics can be used for control of bacterial growth in Chlorella cultures. However, we have not observed any adverse effects due to bacterial contaminants and have made no attempt to exclude them in PGE operations.

We have not been able to detect the presence of trace gases in our photosynthetic gas exchangers. Toxic concentrations of trace gases are apparently not produced by Chlorella 71105. The Cebus monkey used last year in closed gas system tests exhibited no respiratory difficulties or toxic symptoms.

Of PGE operating variables that we have investigated, the most significant factors affecting algae and O_2 production are light input, the illuminated area to which the algal suspension is exposed, and the nutrient medium feed rate. The significance of the illuminated area is apparently related to the light distribution within the algal suspension, while the specific growth rate of the alga is governed by the nutrient medium feed rate in conjunction with the light provided. Within the limits investigated, agitation, medium concentration, and

CO_2 concentration do not appear to affect algae or O_2 production significantly. Although the chemical composition of Chlorella is affected by high light intensity, an equivalence of 1 liter of O_2 per gram of dry algae was found to hold for a wide range of light inputs.

In PGE design, we have found that an apparatus allowing continuous culture provides a relatively constant environment for the alga. Moreover, the various processes can be easily automated. Although a wide variety of construction materials are otherwise suitable for PGE fabrication, some are toxic to the alga and caution must be used in selecting them. Gas leakage is minimized by using welded construction, the use of as few dynamic gas seals as possible, and O-ring static seals. The hermetic integrity of the system and the reliability of gas flow measurements and gas analyses must be assessed through material balances.

SECTION VI

CONCLUSIONS

Page intentionally left blank

SECTION VI

CONCLUSIONS

The conclusions given here are considered to be valid only with regard to the equipment used in the factorial series and over the range of variables tested in the series. Where conclusions have more general significance, the subject has been treated in the discussion.

From the data obtained, the following conclusions may be drawn:

1. Light input significantly affected O_2 and algae production. Light input also significantly affected the chlorophyll, nitrogen, and carbon contents of the algae produced.

2. Algae production, O_2 production, and CO_2 consumption increased with increasing light input.

3. Chlorophyll, nitrogen, and carbon contents of the algae decreased with increasing light input.

4. Agitation did not significantly affect any of the above dependent variables.

5. The moisture and ash contents of the algae produced were independent of light input and agitation rate.

6. The assimilatory quotient was independent of light input and agitation rate. On the basis of the chemical composition of algae grown, the assimilatory quotient throughout the factorial series was 0.83.

7. The maximum gas exchange capacity of the PGE was equivalent to the respiratory requirements of 0.14 of a man.

8. The production of one gram of algae (dry) was equivalent to the production of 1.03 liter of O_2.

9. The yield and the chemical composition of algae were determined regularly during the factorial series and were used to check gas exchange rates as determined from gas analyses. The former proved to be less variable and more reliable than the latter.

10. The biochemical composition of Chlorella 71105 was similar to other strains of Chlorella.

11. A mathematical expression has been derived expressing the distribution of light within the algal culture.

Page intentionally left blank

BIBLIOGRAPHY

Page intentionally left blank

BIBLIOGRAPHY

1. Burlew, J. S., Ed. Algal Culture from Laboratory to Pilot Plant, Carnegie Institution of Washington Publication 600, 1953.

2. Gafford, R. D., and Dyer, D. L., "Some Characteristics of a Thermophilic Blue-Green Alga," Science 134:616-617, 1961.

3. Horwitz, William, Ed. Methods of Analysis Association of Official Agricultural Chemists, Eighth Edition, 1955.

4. Kok, B., "On the Efficiency of Chlorella Growth," Acta Botanica Neerlandica 1:445-467, 1952.

5. Lubitz, J., "The Protein Quality, Digestibility, and Composition of Chlorella 71105," Contract AF33(616)-7373, Wright-Patterson Air Force Base, Ohio, 1960.

6. Myers, J., "Study of a Photosynthetic Gas Exchanger as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin," School of Aviation Medicine, USAF, Randolph AFB, Texas, Report No. 58-117, 1958.

7. Myers, J., and Brown, A., Gas Regeneration and Food Production in a Closed Ecological System, National Academy of Sciences - National Research Council Publication 893, 1961.

8. Myers, J., and Graham, J. R., "On the Mass Culture of Algae. II Yield as a Function of the Cell Concentration under Continuous Sunlight Irradiance," Plant Physiology 34:345-352, 1959.

9. Myers, J., and Johnston, "Carbon and Nitrogen Balance of Chlorella During Growth," Plant Physiology 24:111-119, 1949.

10. Phillips, J. N., and Myers, J., "Growth Rate of Chlorella in Flashing Light," Plant Physiology 29:152-161, 1954.

11. Office of Naval Research, "Conference on Photosynthetic Gas Exchanger," Washington, D. C., June 11-12, 1956.

12. Sorokin, C., "Tabular Comparative Data for the Low and High Temperature Strains of Chlorella," Nature 184:613-614, 1959.

13. Spoehr, H. A., and Milner, H. W., "Chemical Composition of Chlorella; Effect of Environmental Conditions," Plant Physiology 24:120-149, 1949.

14. Tamiya, H., "Mass Culture of Algae," Annual Review of Plant Physiology 8:309-334, 1957.

15. West, E. S., and Todd, W. R., Textbook of Biochemistry, The MacMillan Company, New York, p 683, 1952.

16. Zuraw, E., et al, "Photosynthetic Gas Exchanger in the Closed Ecosystem for Space," Contract NASW-95, SPD 60-085, 1960.

17. "An Engineering Approach for a Closed-Cycle Photosynthetic System," P59-047, General Dynamics Corporation, Electric Boat Division, 1959.

APPENDICES

APPENDIX A-I

SUMMARY OF PUBLISHED INFORMATION ON ALGAE AS FOOD FOR SPACE TRAVEL

SUMMARY

1. On permanent space stations and in extended interplanetary travels, it is apparent that waste materials must be reconstituted into useable materials if reasonable weight-volume relationships are to be maintained. Algae with a high nutritional content can be cultured for food in association with waste recovery and photosynthetic gas exchange systems.

2. Algae have been cultured and processed under a wide range of conditions; consequently, the quality of algae has varied. The protein efficiency ratio (PER) and digestibility studies have indicated that algae may be as useful a food source as many current crop plants, Unfortunately, many of the studies have failed to define the precise role and potential of algae as food. For example, the algae used as a food source for rats have not been the sole source of protein; adjustments for differences in digestibility and protein content of algae as compared with control foods were not made. Nutrition studies must be more closely correlated with biochemical analyses.

3. Most of the algal nutrition studies have been conducted with green algae, especially Chlorella and Scenedesmus. There is a need for more adequate study of other algal phyla, especially the brown and red algae, as possible space foods because they may ultimately be needed either to supplement or replace the green algae.

4. There is a lack of definitive studies concerned with the pathologic effects resulting from eating algae. Any ill effects presently attributed to green algae may actually be due to culturing and processing techniques and to contamination of the cultures.

5. Most likely, an extended food chain will have to be developed in conjunction with the algae. Aquatic plants such as Wolffia and small animals like Daphnia and Tilapia that feed directly on microalgae should be incorporated into the food chain, but it must be remembered that the energetic efficiency of the system will be decreased.

6. Algae can be used with hydroponically produced crop plants, and dehydrated and concentrated foods. This possibility must be investigated further, since space travel will probably depend upon these methods of food production.

7. Culturing and processing methods for the production of microalgae must be improved before a suitable diet that is largely algae, not merely 10% or 20%, can be produced.

8. The evidence presented shows that algae are potentially useful as a prime food source for space travel. The added advantages of using algae for photosynthetic gas exchange and waste recovery make algae an indispensable part of the closed ecosystem for extended space exploration.

INTRODUCTION

A renewed interest in agricultural science has been created by the current population explosion and man's desire to venture into space. Increased crop production and the search for new food sources are receiving the major emphasis for underdeveloped countries, but even the best agricultural techniques can only be expected to provide a maximum finite yield per unit area.

Since the dream of economically growing plants hydroponically has not been realized, the quest for new food sources has continued and led to an evaluation of the conspicuous aquatic plants, the algae. This paper evaluates algae as a potential food source for man, especially for space exploration.

Although no terrestrial mammal, including man, has lived exclusively on an algal diet (Rich et al, 1961), there are many large aquatic filter feeding animals which subsist largely if not exclusively on phytoplankton. Unavailability of algae in a given environment or difficulty in procurement may account for algae not being exploited as food by terrestrial mammals. It is theoretically possible for a land mammal to subsist on algae exclusively. This paper explores the possibility of algae being used either as food or as a food supplement.

This paper reviews the state-of-the-art of algae as food since the reviews of Lavery and Tischer (1958), Edwards (1958), Gini et al (1960), and others.

HISTORICAL BACKGROUND

A short review of the current uses of algae by man has been presented by Black (1958). The Red Algae include the agar (bulk laxative, textile sizing, gel in desserts, and nutrient media) and carragheenin (pudding, dessert), producers. Porphyra, which is used as a food in Japan, contains all the essential amino acids, minerals, and vitamins; high biological value protein comprises 20-40% of the total dry weight. Quantitative figures on the availability of red algae in the ocean are not known.

The brown algae include Ascophyllum and Laminaria, which are dried to make meal; Laminaria and Macrocystis are sources of algin. The protein content is seasonal and dependent upon the available nitrate.

The brown algae are rich in the carbohydrate, mannitol. Mannitol in large amounts acts as a laxative, however. Although Vitamin B_{12} is found in red and brown algae, the actual synthesis is presumably due to commensal bacteria (Erickson, 1953, Erickson and Banhidi, 1953).

The green algae have always been important in the food chain but only recently have been investigated as a possible source of food for man. The alga Chlorella has received the most attention.

An extensive review of the status of Chlorella as a food source and culturing techniques, especially mass culture, appeared in 1953 (Burlew, 1953). Fisher (1955) listed the advantages of mass-cultured algae:

1. The calculated annual yield of algae is higher than that of conventional crops.

2. Basically, algae require only light, water, CO_2, and inorganic salts for growth.

3. Algae yield more protein, fat, and vitamins than all conventional crops except certain seeds.

4. Most of the solar energy absorbed by unicellular algae is converted to useful food; there is little inedible waste.

5. Algae can be grown in areas otherwise agriculturally useless. They can tolerate high temperatures, and little water is wasted in cultivation.

6. Algae are adaptable to an industrial approach for mass cultivation and processing.

It has been calculated that an acre of mass cultured algae can produce up to 40 tons of dried food per year containing about 20 tons of protein (Anon., 1959; Black, 1958), compared to an acre of soybeans

that will produce about 0.75 ton of dry material containing 0.25 ton of protein per year (Anon., 1959). An article in Nutritional Reviews (Anon., 1959) proposed that the extravagance in terms of equipment and facilities for algae farming is compensated by the advantage of using the algae directly as food by humans. Vegetable foods are now raised primarily to feed livestock. The efficiency of conversion of plant material to meat is less than 30%; usually the efficiency range of the conversion is 5-10%. The efficiency of food conversion decreases proportionally to the length of the food chain. At the present time, unfortunately, open pond mass culturing of algae is impractical due to difficulties in maintaining a proper nutrient medium and in harvesting the algae. Contamination of pond cultures would also be a continual problem.

It is difficult to determine who first visualized the use of green plants for photosynthesic gas exchange. Certainly Joseph Priestly deserves much of the credit. The primitive people who first used algae directly for food set the stage for the use of algae as food in space.

SPACE LOGISTICS

Whisenhunt (1961) emphasized that all space systems must be simple, redundant, and of such a nature that the crew can perform all repairs. Thompson (1960,b) stated that all systems must be 100% efficient in providing the proper cabin environment. Welch (1960) has indicated that physical work, psychological stress, and work under conditions of weightlessness must be studied further before the exact requirements of the space traveler can be established. Whisenhunt (1961) calculated that one man would require a total of 8.3 lbs/day of food, water, and oxygen.

Certainly, as Hanson (1960) stated, the space craft must simulate man's terrestrial environment; the means of simulation will depend upon the duration of the trip. Hanson insisted that the solutions to space feeding problems are a long way off; for example, he emphasized that Chlorella is not a food despite its nutrient content. While hibernation as proposed by Hock (1961) is a possibility, current research must proceed on the assumption that the astronauts will be active during flight. Taylor (1958) has suggested the use of high caloric substances other than carbohydrates and fats, such as jet fuels, but the conversion of such materials to food would require major technical advances. Thompson (1960,b) estimated that it would require 200 tons of propellants to transport one ton of food and drink to the moon. Gaume (1958) established criteria for the selection of food for circumplanetary and interplanetary travel, and concluded that dehydrated and concentrated foods meet the requirements of easy preservation, low waste residue, and easy preparation. Algae, furthermore, could be used in conjunction with dehydrated and concentrated foods.

THE NUTRITIONAL VALUE OF ALGAE

Edwards (1958) has summarized the information available up to 1958 dealing with the nutritional value of algae. She stated that algae may be expected to be used as either a garnish or a food. Vitamins and the sulphur-containing amino acids, for example, might have to supplement the algae. According to Edwards, no adverse affects would be expected from algae cultured and processed under proper conditions. Two comprehensive bibliographic reviews concerning the use of algae as food and the role of algae in space travel have been compiled by the U.S. Army Quartermaster Command (Lavery and Tischer, 1958; Gini *et al*, 1960).

Lubitz (1961) reported that *Chlorella* contains all the essential amino acids but contains insufficient amounts of the sulphur-containing amino acids; 1.15% of the total protein consisted of methionine; cystine and cysteine were not detected and hence must be present in trace amounts, if present at all. W. C. Rose (1938) has shown that cystine can substitute for methionine in the white rat. Unfortunately, the low level of cystine in *Chlorella* will not permit such a substitution. The deficiency in sulphur-containing amino acids in *Chlorella* should not be overemphasized since the corn protein zein which is nutritionally important, according to White *et al* (1954), is also deficient in these same amino acids. Hundley, *et al* (1956) indicated that *Chlorella* contains large amounts of the essential amino acids lysine and threonine. The Hundley group also found that the threonine content of *Chlorella* compared favorably with several animal proteins and was higher than the threonine content of purified soya protein.

Cook (1960) analyzed a 10:1 mixture of *Scenedesmus quadricauda* and *Chlorella spp.* grown in outdoor ponds on sewage and organic wastes. The dried algae contained 41.8% protein, 7.2% carbohydrate, and 27.4% fat. The ash content was high due to the culture conditions. The algae contained 100,000 I.U. per 100 g of carotene as compared with 9,240 I.U. of carotene in spinach, or 43,900 I.U. of vitamin A in beef liver. Liver contains more riboflavin, niacin, pantothenic acid, and vitamin B_{12}, but less thiamine and folic acid than the algae. She reported 39.6 mg per cent of ascorbic acid, which appears low when compared with the 200 to 500 mg per cent found by Morimura and Tamiya (1954). Cook stated that the low concentration of ascorbic acid may be the result of the preparative technique, since 99% of the ascorbic acid was found in the form of dehydroascorbic acid, an oxidation product. It is interesting to note that 102.8 mg per cent ascorbic acid was the highest recorded for orange juice by Booher *et al* (1942). Actually the concentration of ascorbic acid is not low when compared with the 15.6 mg/100g found by Lubitz (1961). Cook's results would probably be modified by improved harvesting and processing techniques.

Milner (1951) cultured *Chlorella* in a medium containing very low fixed nitrogen. The resulting *Chlorella* when dried were 86% lipid and 85% of the fatty acids were unsaturated. All of the essential amino acids

were present and represented about 42% of the total protein present. Thomas and Krauss (1955) tested the effects of nitrogen, potassium, phosphorus, and cellular carbohydrate levels on protein synthesis. They found that the nitrogen level in the cell could be modified, but the relative proportions of the nitrogen fractions remained fairly constant. For example, the protein nitrogen represented 90-95% of the total cell nitrogen regardless of culture conditions. Thomas and Krauss concluded that nitrogen, phosphorus, potassium, and cellular carbohydrate levels are all essential for protein syntheses but that only nitrogen is needed to maintain preformed protein.

Lubitz (1961) analyzed the major components of Chlorella. His results can be summarized in the following table:

Component	% Component Present		
	Algae	Casein	Egg Protein
Protein	55.5	83.1	86.8
Crude fat	7.5	0	0.85
Carbohydrate	17.8	0.68	2.2
Ash	8.25	1.97	3.30
Moisture	7.0	11.20	3.20
Crude fiber (cellulose and hemi-cellulose)	3.1	0	0

He concluded on the basis of these contents that a man would require the following amounts of algae per day to satisfy the minimum daily requirement:

Thiamine	200 g
B Carotene	6 g
Ascorbic acid	500 g

Man requires 25.5 Cal/Kg body weight/day for basal metabolism (Spector, 1956); additional caloric requirements are dependent upon work performed. Thus a 150 pound man requires 1720 Cal/day for basal metabolism. According to Lubitz (1961), Chlorella has 3.3 available Calories per gram, consequently 600 grams would provide 2000 Cal.

EXPERIMENTS WITH ANIMALS

Hayami and Shino (1958) tested decolorized (solvent extracted) and untreated dried Chlorella as a food source for rats. While the decolorized alga was better than the untreated in promoting growth, neither was as good as a casein diet. Prosky and Karinin (1960) used weanling rats and found that Chlorella were 70% digestible as compared with 95% for the casein control. When algal protein constituted 20% of the diet, the growth rate for these animals equaled that with the casein controls. Prosky and Karinen also found that the calcium-to-phosphorous ratio was important in the algal diet, and that a mineral deficiency induced in the algae could be reversed by adding a salt mixture to the diet to restore the proper salt balance. Thus it is important in evaluating the results of a given algal diet growth experiment to know whether or not a mineral deficiency is involved.

Nakamura and Yamada (1960) also found that the growth-promoting effect of decolorized Chlorella was better than that of the non-decolorized material. These authors found that a diet consisting of wheat and 10% decolorized Chlorella yielded 50% better growth than a skim milk diet. With the decolorized algal diet, however, methionine was required. Nakamura and Yamada have proposed that the role of methionine is to improve the assimilation of nitrogen. No appreciable difference was observed in chicks raised on either an assorted diet or on wheat, regardless of whether algae were added or not. In older birds, egg production increased 5% when algae were added to the diet, but the eggs were smaller than the eggs from control birds. No statistical evaluation of the effect was made.

In the experiments by these Japanese scientists, the increased absorption of the decolorized algae may have been due to the better digestion resulting from the breaking down of the algal cell wall. Unfortunately, the chemical composition (protein content, for example,) of the solvent-extracted algae was not given. To test the effects of algae on growth, the algae should have been the sole source of protein except for the addition of known amounts of specific amino acids. The amount of the algae present in a diet is critical in a growth experiment. For example, if an excess of algae is added, a deficiency of an amino acid will be masked by the bulk of the additional algae. Thus a 10% algal diet yields more information than a 30% algal diet, because the true nutritional value of the algae will be determined and can then be compared with other foods. Results from nutritional studies should be correlated with biochemical analyses in order to establish the identity of the deficient element. In any case, all adjustments in amounts of given substances in the diets should have been accounted for. In the design of growth experiments it must be remembered that: 1) corrections must be made for differences in digestibility when food sources such as skim milk and algae are compared, and 2) the longer the growth experiment is continued, the better any given protein source will appear, as the result of the leveling off of the organisms' growth rate. In general, the Japanese studies cited in this section do not appear to have considered a number of factors.

Cook (1960) and Cook and Lau (1961) studied the nutritive value of waste-grown algae. Both studies used a mixture of Scenedesmus and Chlorella. Cook determined the protein efficiency ratio (PER) for the algae. (PER is the grams of weight gained by the test organisms per gram of protein consumed). The PER for algae was 1.62 while the PER for casein was 2.31. When a diet in which casein furnished 75% of the protein was combined with algae, the PER equaled that of casein alone. Since both casein and algae contain low amounts of methionine and cystine, casein is not the best source of supplemental protein for an algal diet. Both autoclaving and cooking the algae for two hours decreased the PER; cooking for 30 minutes raised the PER and increased the digestibility of the algae. The autoclaving made the algae less acceptable to the rats so that food intake was decreased. Cook and Lau (1961) concluded that waste-grown algae cannot be expected to meet all the nutritional needs of man.

Tamiya (1961) and the Japanese group studied vacuum dried, spray dried, freeze dried, ethanol treated, methanol treated, and blanched algae. They determined that different chemical and nutritive values can be obtained when different culturing and processing techniques are used. It was concluded further that nutritive values will vary among different algal species. The blanched algae showed by an in vitro method a digestibility value of 66.2, compared with 91.7 for casein. The freeze dried algae were next highest with a value of 55.3.

The use of the Melnick (trypsin digestion) method of determining digestibility of various algal samples should not be relied upon until an adequate in vivo reference point is established. Unfortunately, the blanched algae which showed the highest in vitro digestibility were not used in the nutrition studies. It is obvious that a substance must be digested before it can have nutritional value. In the Japanese work, the various diets do not appear to have been adjusted to equivalent protein content.

For a valid comparison of growth rates, the diets should have been adjusted, not only in terms of protein content, but also in terms of digestibility. Furthermore, weanling rats were not used in the experiments; older animals are less reliable for establishing growth indices. The most one can say is that in these experiments the rats survived and grew on the various diets.

The Tamiya group also conducted experiments with the water-flea, Daphnia, which feeds on algae. Semi-micro Kjeldahl analyses were made of the nitrogen content of the dried Daphnia. From these analyses, a nitrogen conversion efficiency of algal nitrogen to Daphnia nitrogen was calculated. This nitrogen conversion efficiency was considered representative of protein conversion efficiency. Such an assumption is unwarranted because Daphnia, like other crustaceans, possesses a chitinous exoskeleton; chitin is a nitrogen containing carbohydrate composed of repeating units of glucosamine. Therefore, a great deal of the nitrogen found in Daphnia is non-proteinaceous. Also, when whole Daphnia are analyzed, the intestine is filled with algal

material, which may confuse the results significantly. Any growth studies involving Daphnia would be improved by the use of cohorts (offspring produced by a single female at one time) especially when few Daphnia are studied. The use of cohorts would ensure, as much as is possible, the use of homogeneous population for growth studies. In experiments with rats, semi-homogeneity is frequently achieved by using inbread animals of a given age or size.

Fink (1958) used the alga Scenedesmus as the sole source of protein (except for the obligatory supplementation with brewer's yeast) in rat growth experiments. Algae, milk, and egg protein yielded nearly equal growth in weanling rats; after 100 days of growth, the algae appeared to support better growth than the other protein sources. No amino acid supplementation was required with the algal diet. (This is surprising, since one would expect the algae to be deficient in the sulphur-containing amino acids.) The skim milk protein diet required cereal protein supplementation. The rats showed no ill effects after eating algae for 240 days.

Lubitz (1961) used Chlorella 71105 in studies of digestibility, PER, and growth of rats. This particular strain was selected by Benoit, et al (1960) because it exhibited the best combination of desirable characteristics for the closed-cycle application.

Lubitz calculated the coefficient of digestibility for the algae protein as 86%. This compares favorably with beef liver, whole wheat, rolled oats, meat scraps, and fish meal. Using digestibility and protein content information, suitable adjustments were made in test diets. The total carbohydrate was 72% digestible. The crude fat and crude fiber were 93% and 15% digestible, respectively. Lubitz compared the PER of freeze dried Chlorella, Chlorella plus 2% L-methionine, casein, and defatted egg protein. The L methionine was added to the algae to bring the level of the sulphur-containing amino acids up to the level found in a 10% casein ration, which doubled the amount of L-methionine found in dried Chlorella alone. The PER of Chlorella compares favorably with casein, beef liver, and hamburger and is definitely better than vegetable and cereal proteins. Lubitz's study showed that freeze dried Chlorella will support adequate growth of young rats.

The Tamiya group experimented with algae as a food supplement for humans. They calculated the "apparent" and "true" digestibility. The true digestibility was 75%. McDowell et al (1960) carried out preliminary tests on the acceptibility, digestibility, and toxicity of microalgae to humans. They found gastrointestinal symptoms developed on the first day of each algal experiment, although 100 grams per day of algae were accepted. At a 200-gram level, the gastrointestinal symptoms increased, and the algae diets were distasteful to the participants. Two subjects tried a maximum of 500 grams for two days and developed severe gastrointestinal symptoms, mild malaise, and headache. Physiological and chemical laboratory tests showed no other toxic effects related to eating algae.

Jorgensen and Convit (1953) reported that a soup made from a heterogeneous plankton population had health-promoting effects on leprous patients.

Malkin (1961) and Myers (1961) considered the high protein content (50%) of algae to be an important problem in a diet for humans. Malkin (1961) added that the astronaut on an algal diet will consume large amounts of "algulose".

ALGAE TOXICITY

For assays of the toxicity of algae, pure cultures or selected mixed cultures must be used, since some common contaminants of algal cultures may be toxic. For the purpose of using algae as food in space travel, we should know whether the toxin is inherent in the organism, due to decay or to culture or processing conditions, or the result of mutation within a given culture (Anon., 1961).

Milner (1951) expressed concern regarding an antialgal substance called Chlorellin which is reportedly produced by Chlorella. Chlorellin was reported to inhibit growth of Chlorella and other algae (Pratt and Fong , 1940). The chemical nature of Chlorellin has not been established.

Rose (1960) has reviewed the knowledge of production by various organisms of inhibitory substances which affect other members of their own species. This phenomenon may be much more widespread than previously thought. The inhibition might in some cases be due to parasites specific to particular species. As a problem in culturing organisms, the presence of inhibitory substances may be just as important as nutrient depletion. For example, the net value of conventional crop rotation may be more than replenishment of given nutrients.

Dillenberg and Dehnel (1960) reported the death of dogs, geese, large fish, cattle, and horses due to the blue-green algae Anabaena and Microcystis. The two cases of human poisoning reported by these authors were not fatal. Bishop et al (1959) isolated from Microcystis a fast-killing factor, a cyclic polypeptide, which causes death in 2 to 3 hours. A slow-killing factor has also been isolated (Hughes et al, 1958). The toxic effect of "red tide", which is of algal origin, is well known. Water-soluble toxins produced by algae may be expected to withstand filtration and chlorination as well as alum precipitation.

Ingram and Prescott (1954) and Vinberg (1955) have surveyed the problem of the toxicity of blue-green algae.

Combs (1952) reported experiments in which a 20% level of Chlorella in chick diet showed no toxic effects and promoted good growth of chicks.

There are no reports in the literature on histological studies concerned with the possible ill effects of using green algae as food, but Lubitz (1961) has begun work in this area. Alopecia (loss of hair) appeared in one out of ten Chlorella-fed rats and two out of ten Chlorella plus L-methionine-fed rats. No mites or insects were found associated with the alopecia. Further study of the potential pathological effects of algae should be carried out.

PSYCHOLOGICAL ASPECTS

Finkelstein (1958) has shown that factors such as reduced barometric pressure and the breathing of 100% aviator's oxygen do not influence taste perception. Food discrimination, however, is limited in subjects kept in the dark. Therefore, in proposed space capsules one may reasonably expect the responses of space travelers to various foods to be relatively independent of the cabin atmosphere. Finkelstein emphasized the role of food as prime source of social and personal satisfaction to the astronaut, so the psychological aspects of the use of algae as food must be considered. This is especially true if the food algae are associated with waste reclamation.

SOME FOOD SOURCES OTHER THAN ALGAE FOR THE ASTRONAUT

The duckweed Wolffia has been considered as a possible food source for space travel by Nakamura (1961). Wolffia, unlike Chlorella, contains much starch and according to Nakamura, tastes like cabbage. The vitamins A, B_2, B_6, and C were detected in the plant, with C the most abundant. The analyses of the whole plant showed:

Carbohydrate	60-65%
Protein	8-10%
Fat	18-20%
Minerals	6-8%
Chlorophyll	1-2%

Duckweeds have been studied as photosynthetic gas exchange plants (Ney, 1960). Dr. S. S. Wilks has been mass-culturing Wolffia for photosynthetic gas exchange at the School of Aviation Medicine, Brooks Field, Texas (personal communication). The possibility of increasing the length of the food chain for space travel has been considered by many workers. Myers and Brown (1961) emphasized the need to minimize the mass of organisms required in extending the food chain. Preliminary investigation with the tropical fish Tilapia has been conducted by Lorant (1959). This particular fish grows rapidly, reproduces at the age of 8 weeks, is tasty, and feeds entirely upon algae. It will live on algae which are grown on human wastes. The potentialities of such a food organism as Tilapia are increased when one considers long interplanetary trips, space stations on the moon, etc.

Dr. John R. Olive and his group at Colorado State University have proposed Daphnia as a means of extending the food chain for space travel. The energetics for mass-cultured Daphnia have been reviewed by Slobodkin (1959).

Thompson (1960,b) has considered the use of hydroponics for supplying food for a permanent moon station because of the lack of soil, water, and atmosphere on the moon. Thompson proposed that there would be no need for a transparent covering over the plants, since the moon's day and night equal 14 terrestrial days. His system would depend entirely upon artificial light. A more realistic approach, however, would be to use natural light during the day and auxiliary light at night and locate the station near the poles for longer light periods. The lack of atmospheric screening of irradiation on the moon presents a problem because of the potential hazard to the algae and the men.

Rich et al (1960) evaluated hydroponically grown higher plants for both oxygen and food production in closed ecological systems. Nickell and Tulecke (1960) have mass-cultured several plant tissues. Plant tissue culture should be investigated as a possible food source for space travel. For the space application, only edible portions of the plants would be cultured.

APPENDIX A-2

SUMMARY OF PUBLISHED INFORMATION ON ALGAE AND WASTE TREATMENT FOR SPACE TRAVEL

Page intentionally left blank

SUMMARY

1. In a closed ecological system, the entire waste influent must be treated in such a way as to permit recycling the materials contained in the sewage. It is well established that algae are useful in treating domestic sewage. They thrive on it, and besides treating sewage, the algae can absorb carbon dioxide and supply both oxygen and food.

2. The main advantages of algal systems are relative simplicity and fixed weight and volume regardless of mission duration, but further research is required before the algal system can be made to satisfy the weight-volume requirements of a closed rocket environment.

3. One reasonable approach is the combination of an algal system with an activated sludge unit. Further refinements dealing with light and nutrients must be developed. A method of breaking the sewage down to particles of less than one micron is required. All sewage organisms must be selected on the basis of their efficiency in reclaiming the waste produced in the specific closed environment.

4. The indications are that algae will be important for waste treatment on extended trips and for permanent extraterrestrial installations. Progressive development requires further research into anaerobic, oxidative, aerobic, algal, chemical, and mechanical waste handling techniques. Available evidence, while favoring some systems, does not exclude others. At the present time, it appears that a combination of systems will be necessary. For example, material which is not easily oxidized by microbes may require incineration. Another specific need is for efficient emulsification and degradation of the grease component of sewage.

5. Further studies of the biochemical oxidation of specific chemical wastes and the role of algae are needed. At a later stage, these studies could be applied to the wastes encountered on a space vehicle. The next logical step would then be to select the organisms which would perform waste degradation most efficiently.

6. The role of key elements such as nitrogen in sewage systems must be further elucidated.

7. The biochemical oxygen demand (BOD) and the chemical oxygen demand (COD) are standard measures of sewage strength. In studies of

the kinetics and chemical reactions involved in sewage degradation newer analytical techniques must be employed to implement the BOD and COD determinations.

8. Empirical studies of sewage treatment must be implemented by increased emphasis upon theoretical considerations. Although domestic sewage is a complex aggregate of organic and inorganic materials, it is amenable to theoretical studies.

INTRODUCTION

Man in space will encounter unique sanitation problems, especially on extended trips. The complexity of the problem is engendered by the confined quarters, zero gravity, low weight-volume requirements, and limited power supply. Any waste treatment process must satisfy these fundamental environmental and operation characteristics; the system must be able to process, with complete efficiency, the daily excretory and eliminatory products of a man or men on a stipulated diet.

Whisenhunt (1961) compared the open, semi-closed, and closed environmental systems and concluded that, for any permanent space installation, the closed environment will be necessary. Many new developments will be needed to implement this type of environment.

Since the space traveler must have all his needs satisfied by equipment contained within the space craft, the problems of weight and bulk become especially important. For example, Thompson (1960, a) has stated that a man produces 8 lbs 2 oz of wastes per day, which must be matched by ingestion of food, oxygen, and water. Fecal weight alone (4-6 oz/man/day) on a three year space trip would ultimately equal the weight of two crew members. Jettisonning of wastes is not an acceptable solution for extended voyages; additional substance would be required to compensate for the loss of materials, and the space craft is too small for storing expendable (and heavy) supplies. Is is obvious that the wastes must be treated and recycled to save space and weight. Several means exist.

Chemical-mechanical Methods

Chemical-mechanical waste treatment systems are compact and easy to manipulate and might require little energy for operation. On the disadvantage side, Golueke, et al (1959) have indicated that chemical-mechanical systems would increase in weight and volume in proportion to the length of the voyage, and further, that even with distillation and ion exchange, the solid residues must be filtered out for disposal, storage, or biological treatment. Boiler scale, blow-down, and waste from regenerating chemicals would fill much of the space which would more practically be devoted to control and test equipment.

Biological Methods

In a review of waste treatment for closed ecosystems, Pipes (1961) pointed out one important advantage of biological waste processing:

the retention of a maximum amount of waste in a biologically active form. A biological system employing bacteria and algae could have a constant weight regardless of the trip duration. Since this same biological system is a potential source of food and oxygen, one system could perform three functions - waste removal, food supply, and oxygen supply. (The value of algae as a source of oxygen for space travel has been discussed by Myers (1957), Bates (1961), Bialecki (1961) and others.) The presence of algae in sewage ponds and filters has been observed for many years. General papers have appeared concerning the species involved, but only recently have refinements evolved in the studies of algae in sewage. Ingram (1958) and his group reviewed the literature dealing with the role of algae in sewage treatment. They expressed the view that at that time algae did not appear to be useful for waste treatment in closed ecological systems. They reasoned that, in addition to requiring extensively pre-treated sewage, the algal culture might produce dominating disadvantageous mutations or be destroyed by biological contaminants. Harvesting and processing the algae were also cited as problems. Welch (1960) also favored non-biological systems for conservative waste treatment.

D.D. Chapman (personal communication) and his group are investigating both physical and biological methods for treating wastes. He was very optimistic about the value of biological waste reduction for closed ecosystems. These scientists have developed a microbial population which can subsist on raw human waste. Such waste is 300 times more concentrated than domestic sewage. They constructed a bench-scale plant which operated at 30°C and which, when well aerated, would require only one-half cubic foot of culture volume to process the wastes of a four-man crew. The system as described by Chapman makes no provision for the residue produced by the activated sludge culture.

The number of experimental papers dealing with the potential role of algae in treating wastes in closed environments is limited. This report reviews the status of systems for treating domestic waste with algae and algae-bacteria in closed environments since Ingram's review (1958).

In a biological system, the bacteria would oxidize the wastes; the algae would subsist on these oxidized wastes, carbon dioxide, and other materials produced by the bacteria and man. The algae, besides completing the waste degradation and providing oxygen for aerobic waste digestion, would supply food and oxygen for the crew. Studies on the nutritive value of waste-grown algae have been promising (Cook, 1961, Cook and Lau, 1961). Instead of a single unit, Golueke, et al (1959) combined an algal culture with an activated sludge unit. A method of processing waste-grown algae for human consumption has not been established.

TECHNICAL DISCUSSION OF SEWAGE TREATMENT PRACTICES

Activated Sludge Units

Activated sludge methods in sewage plant operation are well understood. If a completely biological system were to be employed, an activated sludge unit would probably be used in conjunction with algae. It may be possible to scale down high-efficiency activated sludge units for use in closed environments.

A concise description of the activated sludge process was presented by Hatfield (1957). Basically, an aerobic flora grown on organic wastes with repeated aeration and settling produce a biologically active mire. The organisms of the mire degrade the waste organic compounds (the excess activated sludge may be dried or anaerobically digested to produce a gas consisting of about 65% methane and 35% carbon dioxide). The most common organisms in an activated sludge system are bacteria, protozoa, and rotifera. The protozoa, according to Lackey and Hendrickson (1958), probably help maintain the bacterial population in the logarithmic growth phase. Actively growing bacteria apparently oxidize wastes more efficiently.

In order to derive the parameters of an activated sludge process, the nature of the waste to be degraded must first be established.

It is well known that a given waste will require a specific group of organisms for degradation. For example, a specific flora treats phenolic wastes (Hatfield, 1957). More studies which deal with the specific organic constituents present in various wastes are required.

Heukelekian and Balmat (1959) analyzed the seasonal fluctuation of the inorganic and organic constituents of municipal sewage. They classified the total dry solids into the following fractions: 1) settleable, 17%; 2) supracolloidal (0.1-1μ dia.) 12%; 3) colloidal, 7%; and 4) dissolved, 64%. The insoluble material representing about 36% of the total solid matter was composed of 80% organic to 20% inorganic substances. Materials not in solution are less amenable to degradation by organisms.

Grease (composed mainly of saturated glyceride fatty acids plus lesser amounts of unsaturated glyceride fatty acids and unsaponifiable matter) represented 51.5% of the colloidal fraction, 19% of the settleable, and 24% of the supracolloidal dry solids. The percent concentration of the major mineral components was calculated. The settleable fraction was composed of 50.5% silicon, 5-9% iron, calcium, magnesium, and phosphorus. Sulfur was present at a concentration of 0.7%. Phosphorus represented 36% of the mineral weight of the supracolloidal

fraction, while silicon and calcium comprised 15% and 11%, respectively. In recent years, the phosphate content of sewage has increased enormously due to the widespread use of household detergents (Rudolfs, 1947). The colloidal fraction contained 30.9% calcium and 20.5% phosphorus. The dissolved solids were 27% sodium, 24% chlorine, 12% sulfur, and 11% magnesium. A total of 14 anions and cations were quantitatively analyzed. Nitrogenous material was present (in the supracolloidal fraction) in amounts twice as great as that found in the settleable and colloidal fractions.

The amino acids, alanine, aspartic acid, glutamic acid, leucine, phenylalanine, methionine, and valine were present in all fractions. The carbohydrates, lignin, cellulose, hemicellulose, and pectin were identified quantitatively. The settleable fraction contained the highest concentration of carbohydrate material; cellulose and lignin represented 20% of the dry settleable fraction. All chemical analyses must be taken into account in studies with activated sludge cultures if optimum conditions are to be achieved.

The high concentration of grease in domestic sewage presents a problem; it is difficult to degrade grease biologically because of its chemical, semisolid nature. Grease also shields other compounds from action by the sludge flora. The use of biocatalytic additives appears to offer no immediate solution to the grease problem. Pierson et al (1957) tested five well known biocatalytic additives. These additives yielded a higher residual grease concentration, failed to cause grease liquefaction, and failed to show an increase in lipase activity. Biocatalytic additives appear to cause an increase in volatile acids, but it is doubtful that they induce a reduction of volatile solids. As the Pierson group has pointed out, the failure of the additives may have been due to a problem of improper buffering. This hypothesis was supported by one of their experiments which showed that the lipase activity of the additive on olive oil was enhanced by the addition of activated sludge.

The degradation of synthetic sewage seeded with sewage organisms has been studied by Engelbrecht and McKinney (1957) and others. Engelbrecht and McKinney found that differences among various samples of activated sludges could be attributed to organisms which attack specific compounds. It was essentially a case of the substrates selecting the organisms. For example, different flora were associated with sucrose, glucose, and fructose cultures. In addition, these researchers presented evidence which supported the idea that atoms other than carbon in the chain of an organic molecule often interfere with biological oxidation. In one culture the guanidine portion of arginine, for example, was not attacked while the valeric acid fraction was degraded by the sewage flora.

Nitrogen is a key element in biological systems; it has been well studied in metabolism, but much remains to be learned about its role in sewage systems. Symons and McKinney (1958) investigated the role of nitrogen in batch activated sludge processes. In a system with a high

nitrogen content, much bacterial protoplasm and little extracellular polysaccharide was formed; a daily loss of solids prevented a build-up of aeration solids; the amount of active protoplasm declined with time. In a system containing a moderate nitrogen content, the amount of cellular material formed was limited, but the same amount of substrate was processed; the organisms compensated by discharging more extracellular polysaccharide. In the moderate nitrogen system, the level of aeration solids would continue to rise because of the slow decomposition of the extracellular polysaccharide; during long term operation, this system would show a constant amount of protoplasm, an increase in polysaccharide, and a marked rise in the total mass of aerated solids. In a low nitrogen system, there would be little true protoplasm but much polysaccharide formed; since more protoplasm would be degraded by endogenous respiration than would be synthesized, such a situation could only exist in a well established culture suddenly deprived of nitrogen. In a moribund system the total mass of solids increases. Symons and McKinney concluded that the system containing moderate nitrogen was best because there is no daily decrease in the amount of true protoplasm. A study of this sort should be combined with continuous harvest studies such as those conducted by Kountz and Forney (1959).

In a closed ecological system, the entire influent must be biochemically oxidized or degraded in some manner. Kountz and Forney (1959) arranged a continuous operation using dry skim milk solids and measured solid, liquid, and gas effluents. In this manner they controlled the quality and quantity of the influent and avoided a pseudo-equilibrium due to discharging of the activated sludge. They calculated that dry skim milk, which is a useful analog to sanitary sewage, has a 23% residual which is non-oxidizable. Control of the nitrogen content in the activated sludge system might minimize the accumulation of non-oxidizable materials. The total accumulation of non-oxidizable material was calculated to represent 0.6% per day of the total weight of activated sludge in a continuous flow system. This means that some other treatment process would have to be used to degrade the residual. The equilibrium weight of the activated sludge was calculated to be 14 times the weight of the daily influent. This means that a unit would have to contain about 113 pounds of culture in order to handle the body wastes of one man per day (8 lbs 2 oz).

Sewage Strength Determinations

To evaluate the condition of a given sewage sample, two types of sewage strength determinations are usually made: 1) the biochemical oxygen demand (BOD) and 2) the chemical oxygen demand (COD). In Standard Methods for the Examination of Water and Wastewater (Farber, 1960), the role and methodology of both determinations are described. Both methods may be used to determine the efficiency and rate of waste degradation. The sources of the oxygen demand in wastes are grouped into three classes: 1) organic carbon compounds which are degraded by aerobic microorganisms, 2) organic and inorganic nitrogen sources which are oxidizable and also utilized by organisms, and 3) reducing compounds which react with molecular oxygen. The first class of compounds,

which accounts for most of the oxygen demand of domestic sewage, and the second class are determined by the BOD. The third class of compounds may be included in a BOD determination, if the amount of dissolved oxygen in the system initially is ascertained. The presence of slowly oxidizable organic compounds increases the time required for waste stabilization; therefore, the standard 5-day BOD determination will be inaccurate in ascertaining the ultimate carbonaceous demand (L) unless the exponential rate of carbonaceous oxidation (k) is determined for the specific sewage sample under consideration.

When no catalyst is used, the COD test may fail to include some organic compounds which would be accounted for in a BOD determination. On the other hand, the COD includes some organic compounds which are not readily attacked by organisms. The COD will be low for proteinaceous matter, since the amino group does not reduce the dichromate used in the test. The best agreement between BOD and COD will occur when the organic matter is readily attacked by organisms and no toxic substances are present, but the tests cannot be expected to measure the same characteristic in ordinary practice.

Bogan (1958) has proposed that both tests are very limited. He has pointed out that if whole-molecule reactions were involved, then one should be able to calculate one measure from the other. As Farber (1960) has stated, this is possible under certain conditions; but complex wastes do not allow for such reciprocal calculations. Bogan further criticized the BOD and COD methods in that neither method yields specific information regarding the events leading up to oxygen utilization. In both cases, the nature of the residuals is unknown; for example, organic end products could be formed which might be of more concern that the original wastes. Actually the BOD and COD are not designed to give such information, which emphasizes the fact that more sophisticated tests must be used to answer specific questions. To achieve more critical determinations, Bogan used infrared spectroscopy on cell-free extracts for his analyses of the organic residues in activated sludge. Techniques such as infrared spectroscopy should be used more frequently in sewage analyses; however, their use is fraught with problems and they must be judiciously employed.

Theoretical Considerations

While much of the information regarding sewage degradation is empirically derived, there is an increased interest in the theoretical considerations. Bogan (1958) presented the following list of biochemical reactions which take place in waste degradation:

1. Hydrolysis
2. Beta oxidation or degradation of aliphatics
3. Omega or terminal oxidation of the terminal saturated endings on fatty acids.
4. Fission of rings and unsaturated aliphatics

Balmat (1957) analyzed the rate of biochemical oxidation in specific sewage fractions. He investigated the apparent monomolecular reaction in sewage. The reaction may be stated by the equation

$$\log \frac{L_t}{L} = -k_1 t$$

where L = ultimate BOD; L_t = BOD at time t; k_1 = rate constant.

This equation shows the rate of oxidation as proportional to the remaining unoxidized material. As a consequence, either the rate is independent of chemical constitution, or there are processes with different rates which collectively appear as a monomolecular reaction. It was found that during the first five days of biochemical oxidation, the rate for soluble organic matter is 320% higher than for supracolloidal particles, and 150% higher than for colloidal particles.

Unfortunately, Balmat defined a supracolloidal particle in the 1-100μ range, which differs from the range of 0.1-1μ used by Heukelekian and Balmat (1959). Since a Sharples high-speed centrifuge was used for separation in both cases, the actual supracolloidal particles may be assumed to be the same in both cases, so that the two investigations may be compared. When the rate constant is multiplied by the BOD for each sewage fraction, the results range from 3.8 for the settleable to 50 for the soluble fraction. Thus there is a different rate for each fraction. When the results of this multiplication for each fraction were added, the figure of 72.8 compared favorably with the 60.0 calculated for raw sewage. The results of Balmat's work are: 1) the monomolecular reaction for raw sewage is only apparent, 2) for rapid biochemical oxidation, all solids should be reduced to 1μ or less in diameter. Studies dealing with biochemical oxidation, chemical content, and particle size relationships of raw sewage have yielded useful information which can be applied to closed environments.

Gaudy, et al (1960) have concentrated on the organisms involved in sewage treatment. The modifications and the diverse environmental conditions imposed upon activated sludge units make each unit unique. The biological principles involved in each case are the same, however. The kinetics of batch growth of bacteria can be expressed as:

$$\frac{dN}{dt} = KN \qquad (K = \text{growth rate constant})$$

This equation shows that the change in population number ($\frac{dN}{dt}$) is directly proportional to the number present. In a continuous flow system, with constant dilution, the equation becomes:

$$\frac{dN}{dt} = KN - (Q/V)\, N$$

where Q/V = ratio of inflow (Q) rate to the volume (V) of the container. Once a steady state is achieved, dN/dt is zero so that K = Q/V; i.e., K is a function of the flow and equal to the reciprocal of the detention time or mean residence time, T = V/Q. If Q/V is greater than K, solids will be diluted out. If Q/V equals K, the system will not respond to an increase in supplement. If K_{max} = Q/V, the sludge must be recycled since it is not possible to increase the growth rate of the organisms. K can be externally limited by having all essential nutrients present in non-limiting concentrations except one. Internal control could be exerted by means of a photocell or solenoid, for example. If a limiting nutrient is used,

$$K\ (C_n) = Q/V;$$

that is, K is a function of the controlling nutrient concentration. In a continuous flow system, the number and different proportions of given organisms in a mixed culture can be controlled. Gaudy et al refined their experiments with a continuous flow system by using a synthetic waste which included glucose and NH_4Cl seeded with domestic sewage. These experiments showed that it is possible to have a controlled activated sludge unit containing a specific population and density. The Gaudy experiments should be combined with screening experiments to select the best organisms to attack wastes produced in a closed ecological system.

Sewage Pretreatment

Information derived from stabilization ponds or sewage oxidation ponds (Fitzgerald and Rohlich, 1958; Hermann, 1961; Hermann and Gloyna, 1958) can ultimately be correlated with activated sludge studies. Stabilization ponds can be operated in two ways: aerobically and anaerobically. Aerobic operation is preferred because 1) the detention time is less and 2) odor is controlled. The size of the pond in relation to flow rate and pond depth are important. According to Oswald (1960), 35 to 55 square feet of pond area containing algae of 4% efficiency, exposed to sunlight, produces enough oxygen to oxidize the waste of one person.

It is not proposed to consider stabilization ponds as such in sewage pretreatment for closed environments; however, such studies will implement other work dealing with biological pretreatment of wastes. Stabilization ponds have shown conclusively that algae will thrive on sewage wastes and effectively reduce the BOD and COD.

Instead of combining an aerobic activated sludge unit with an algal system, it might be possible to use an anaerobic system to pretreat the sewage produced in space travel. Lackey and Hendrickson (1958) have reviewed the status of anaerobic sewage digestion. They pointed out that an anaerobic system degrades sewage as completely as an activated sludge unit but takes longer. Anaerobic organisms are fewer in number of species as well as numbers of organisms in both nature and anaerobic digesters. Although the anaerobic flora are implemented by

facultative organisms including protozoa, the population in an anaerobic digester is very small compared to activated sludge. Therefore, the actual sewage digestion must be accomplished by fewer organisms.

Lackey and Hendrickson proposed that a major problem of anaerobic digestion is the lack of sufficient protozoa to maintain the anaerobic bacteria in the logarithmic growth phase. Experiments have shown that facultatives generally prefer an aerobic environment. The protozoa, according to these authors, may prefer the oxygen and food sources found in the anaerobic environment. Regardless of preferences, the organisms would be expected to exploit the anaerobic environment to the maximum. Since the number of organisms is small, the environment itself must be limiting. Growth limiting substances such as ethyl alcohol and lactic acid are produced in the anaerobic system. Therefore, it appears more reasonable to conclude that the organism-environmental interactions existing in an anaerobic system are not conducive for the production of high numbers of organisms. In addition, anaerobic organisms use little of the available energy present in organic molecules. For example, the anaerobic glycolysis of glucose to lactic acid yields 36 kg cal per gram mol compares to the 686 kg cal per gram mol derived from the complete oxidation of glucose (Baldwin, 1959). McKinney and Conway (1957) proposed that the anaerobic system would be just as efficient as activated sludge if the proper nutrients were supplied. They recommended the addition of nitrates and sulphates to a proper biologically flocculated system. The effluent would, of course, be quite turbid due to bacteria. These authors based their speculation upon the energy yields of various hydrogen acceptors, arranged in order of descending energy yields: dissolved oxygen, nitrates, sulfates, oxidized organic matter, and carbon dioxide. Since they recommended nitrates and sulfates which yield 2.5 and 4 atoms of oxygen per molecule respectively, it appears that these authors are trying to provide oxidative degradation in the absence of aeration.

Thus for closed environments there are three major approaches to biological waste treatment: 1) an aerated system in which molecular oxygen is provided, 2) an oxidative system in which various hydrogen acceptors in the form of highly oxidized compounds are present, and 3) a system in which compounds are degraded in the absence of oxygen. These systems may have to be combined for maximum efficiency of waste treatment in a closed ecosystem. It may be that under closed ecological conditions, an anaerobic system may prove to be the most practical (for a permanent moon station, for example). At the present time, the activated sludge and oxidative degradation appear to be more promising than their anaerobic counterpart. If a biological pretreatment proves to be impractical, then chemical and mechanical methods must be employed (see Ingram, 1958).

All biological methods produce a residual sludge (called humus in nature) which must be processed further. Hence, physical methods will have to be used to complete the process. These include drum and spray drying, dry combustion, Kjeldahl combustion, and Zimmerman wet combustion (Zimmerman, 1960).

Sewage Treatment by Algae

Clausen (1958) described the changes which occur in ponds containing settled sewage. Retained sewage becomes septic, darkens, and yields a disagreeable odor. Weeks later, after undergoing anaerobic decomposition, the pond becomes odorless, green, and the amount of colloidal matter is reduced. The septic stage can be eliminated by inoculating the sewage with algae which have been previously grown on sewage. This shows that either the sewage does not have to be preconditioned or that the conditioning proceeds rapidly enough to support algal growth. Clausen described the succession of indicator organisms in the sewage pond. (Indicators are those organisms which are characteristic of a given environment.) It is apparent from the studies of Oswald (1957), and Gaudy et al (1960) that this succession can be controlled. The value of phagocytic algae such as Ochromonas and Euglena for waste treatment may be inferred from various studies (Huntner, et al, 1953; Provasoli and Pintner, 1953).

Oswald et al (1957) calculated that the light energy conversion efficiency of Chlorella and Scenedesmus in sewage is 10 to 12%. Sewage grown Chlorella can produce more oxygen than is necessary to satisfy the BOD (Oswald, 1960). Oswald used sterilized waste and showed that bacterial action is necessary for sewage to support algal growth.

Golueke (1960) conducted experiments with Chlorella, Scenedesmus and a mixed population of bacteria grown in continuous, automatic growth units. He studied the ecological interrelationships in such a biotic community, and as would be expected, found that certain combinations of conditions favored one organism over another. For example, Scenedesmus was favored by CO_2 concentrations in sparged gas greater than 1%, long detention periods, high light intensity, and long periodicities (light duration). Chlorella was favored by completely opposite conditions. A three day detention period produced the highest cell concentration.

In an integrated system, bacteria absorb oxygen and degrade organic molecules to small moieties and CO_2. Algae absorb CO_2 and small moieties and produce oxygen. Both the organic molecules and small moieties contribute to the BOD. Algae require light; bacteria and sewage solids can absorb light.

Oswald (1960) found that the efficiency of an algae culture was increased by increasing temperature, sewage strength, and CO_2 (in the sparged gas); decreased efficiency occurred when the light intensity, light duration, and detention periods are increased. Because of these observations, Oswald proposed that a cyclic depression occurs in a system containing algae and bacteria.

The high pH resulting from rapid photosynthesis may have yielded conditions unfavorable to bacterial oxidation. The lack of oxidized material reciprocally depressed algal growth. Thus cultures with short detention periods and/or brief periods of illumination did not exhibit the wide pH range, but the number of algal cells decreased. Such a culture under conditions of continuous dilution is unable to compensate for the loss in cell numbers because of depressed photosynthesis. Oswald found that compounds such as CO_2 and NH_3 increased in propor-

tion to the BOD of a system. Sewage with a high BOD can be expected to have a lower pH and thus favor bacterial growth. High bacterial populations, furthermore, absorb some of the light required by the photosynthetic members of the culture.

One finding of Oswald's study was that the highest photosynthetic efficiency did not satisfy the BOD. To attain the greatest waste recovery, it is necessary to have the highest possible numbers of algae present under the existing nutrient conditions. Culture productivity (algal concentration x photosynthetic efficiency) must be analyzed to meet the demand of the particular system.

Meffert (1955) showed that algae can subsist on a 30% CO_2 70% methane mixture. Both of these gases are common in anaerobic sewage systems. Bogan (1961) proposed that algal systems are very economical for treating domestic sewage.

Zuraw et al (1960) studied the effects of urine on the growth of Chlorella 71105. Dilutions of 5 to 10% of filtered urine in water supported growth equally as well as standard medium. Urine added to standard medium (5 to 20%) also compared favorably with the standard medium. Experiments conducted in a four-liter vessel containing 20% urine in tap water produced growth equivalent to the standard medium for 24 hours, but the culture density slowly decreased thereafter. The experiments show that it is feasible to use urine as a nutrient source for algae, but pretreatment of the urine may be required.

Studies of a number of selected urine components were also conducted. Sodium chloride was tolerated up to 9 gm/L, which is approximately equal to half the concentration normally found in urine. Allantoin and hippuric acid were not toxic at levels generally present in urine. One gram per liter concentrations of creatinine and creatine stimulated algal growth.

The most comprehensive account of the use of algae for waste recovery in closed environments has been presented by Golueke (1959). He calculated that 7.4 gallons of algae suspension would be required to treat the urine wastes of one man. Fresh urine surpassed all other nitrogen sources for algae growth in continuous or semi-continuous cultures. Golueke integrated an activated sludge unit with an algal growth unit; this two-liter combination treated 1-1.2 grams (dry weight) of fecal solids per day; thus 13.6 gallons would be required to treat the 26 grams of feces (dry weight) produced by a 145-pound man per day. The 13.6 gallons (114 pounds) could probably be reduced to 3.42 gallons. If the algae and bacteria were mixed in one unit, there would be a turbidity problem. The total weight of the integrated unit plus associated components would be 304-411 pounds per man according to Golueke.

In recent press releases, a sewage-grown algae-bacteria complex has been proposed as a "fuel cell" battery, especially under conditions where little power is required over extended periods of time. The catalysis of the raw sewage by the bacteria would produce an electric current; the photosynthetic activity of the algae would regenerate the fuel. Preliminary experiments on such a "bio-battery" have been conducted by G. H. Rohrback and F. D. Sisler (Witkin, 1961).

BIBLIOGRAPHY

FOR APPENDIX A

1. Anonymous, "Algae as a Source of Human Food," Nutrition Reviews, Vol 17, pp 238-240, 1959.

2. Anonymous, "Toxic Waterbloom (Algae)," Nutrition Reviews, Vol 19, pp 145-146, 1961.

3. Baldwin, E., Dynamic Aspects of Biochemistry, Cambridge University Press, London, 1959.

4. Balmat, J. L., "Biochemical Oxidation of Various Particulate Fractions of Sewage," Sewage and Industrial Wastes, Vol 29, pp 757-761, 1957.

5. Bates, H., "Recent Aspects in the Development of a Closed Ecologic System," Aerospace Medicine, Vol 32, pp 12-24, 1961.

6. Benoit, R. J., Trainor, F., Bialecki, A., Selection of an Alga for the Photosynthetic Gas Exchanger, WADC Technical Report No. 60-163, Wright Air Development Division, Wright-Patterson Air Force Base, Ohio, 1960.

7. Bialecki, A., "Feasibility and Logistics of Recoverable Oxygen Systems," to be published in the 7th Annual Proceedings of the American Astronautical Society, 1961.

8. Bishop, C. T., Anet, E. F. L. J., Gorham, P. R., "Isolation and Identification of the Fast-Death Factor in Microcystis aeruginosa NRC-1," Canadian Journal of Biochemical Physiology, Vol 37, pp 453-471, 1959.

9. Bogan, R. H., "Biochemical Degradation Products - A New Dimension in Stream Pollution," Sewage and Industrial Wastes, Vol 30, pp 208-214, 1958.

10. Bogan, R. H., "Utilization of Algae as an Aid in Sewage Nutrient Removals," Engineering Bulletin, Purdue University, Vol XLV, pp 68-80, 1961.

11. Booher, L. E., Hartzler, E. R., Hewston, E. M., A Compilation of the Vitamin Values of Foods in Relation to Processing and other Variants, U.S. Dept. of Agriculture Circular No 638, Washington 25, D.C., 1942.

12. Black, W. A. P., "The Algae," Processed Plant Protein Foodstuffs, Altschul, A.M., Ed., Academic Press, Inc., New York, 1958.

13 Burlew, J. S. Ed., Algal Culture from Laboratory to Pilot Plant, Carnegie Institution of Washington Publication 600, 1953.

14. Clausen, H. T., "Some Observations on the Action of Algae on Sewage in Ponds," Inst. Sewage Purif. J. Proc., Pt. 3, pp 345-348, 1959.

15. Combs, G. F., "Algae (Chlorella) as a Source of Nutrients for the Chick," Science, Vol 116, p 453, 1952.

16. Cook, B. B., "The Nutritive Value of Waste Grown Algae," 88th Annual Meeting of the American Public Health Association, Inc., San Francisco, Calif., 1960.

17. Cook. B. B., Lau, E. W., "The Protein Quality of Waste-Grown Green Algae Alone and in Combination with Cereal and Milk Proteins," Fed. Proc., Vol 20, p 371, 1961.

18. Dillenberg, H. O., Dehnel, M. K., Canad. Med. Assn. J., Vol 83, p 1151, 1960. Cited in Nutrition Reviews, 19, pp 145-146, 1961, Original reference not seen.

19. Edwards, G. P., "Report on the Culture of Algae," Engineering Biotechnology of Handling Wastes Resulting from a Closed Ecological System, Appendix 2 Report No. TR58-148 ASTIA Document No AD162 277, December, 1958.

20. Engelbrecht, R. S., McKinney, R. E., "Activated Sludge Cultures Developed on Pure Organic Compounds," Sewage and Industrial Wastes Vol 29, pp 1350-1362, 1957.

21. Ericson, L. E., Lewis, L., "Vitamin B_{12} Factors in Marine Algae," Archiv. Kemi, Vol 6, pp 427-442, 1953.

22. Ericson, L. E., Banhidi, Z. G., "Bacterial Growth Factors Related to Vitamin B_{12} and Folic Acid in Some Brown and Red Seaweeds," Acta. Chem. Scand., Vol 7, pp 167-172, 1953.

23. Farber, L., Ed. Standard Methods for the Examination of Water and Wastewater, American Public Heath Association, Inc., New York, 1960.

24. Fink, H., "On the Protein Quality and the Liver Necrosis Preventative Factor of Unicellular Algae," Transactions of the Conference on the Use of Solar Energy - The Scientific Basis, Vol IV, Chap 7, The University of Arizona Press, Tuscon, Arizona, 1958.

25. Finkelstein, B., "Feeding Crews in Air Vehicles of the Future," Food Technology, Vol 12, pp 445-448, 1958.

26. Fisher, A. W., Jr., Burlew, J. S., "Nutritional Value of Microscopic Algae," Algal Culture from Laboratory to Pilot Plant, Carnegie Institution of Washington Publication 600, pp 303-310, 1953.

27. Fisher, A. W., Jr., "Engineering for Algae Culture," Proc. of the World Symposium on Applied Solar Energy, pp 243-253, 1955.

28. Fitzgerald, G. P., Rohlich, G. A., "An Evaluation of Stabilization Pond Literature," Sewage and Industrial Wastes, Vol 30, pp 1213-1224, 1958.

29. Gaudy, A. F., Jr., Engelbrecht, R. S., DeMoss, R. D., "Laboratory Scale Activated Sludge Unit," Applied Microbiology, Vol 8, pp 298-304, 1960.

30. Gaume, J. G., "Nutrition in Space Operations," Food Technology, Vol 12, pp 433-435, 1958.

31. Gini, B., Enright, J. J., Byrne, A. F., Burton, T., Strietelmeier, D. M., Koch, R. P., "Space Feeding: A Closed Ecological System for Extended Travel. A Review of Pertinent Literature,". Quartermaster Food and Container Institute for the Armed Forces Library Bulletin No 2, Chicago, 1960.

32. Golueke, C. G., "The Ecology of a Biotic Community Consisting of Algae and Bacteria," Ecology, Vol 41, pp 65-73, 1961.

33. Golueke, C. G., Oswald, W. J., McGauhey, P. H., "The Biological Control of Enclosed Environments," Sewage and Industrial Wastes, Vol 31, pp 1125-1142, 1959.

34. Hanson, S. W. F., "Some Problems of Spaceflight Feeding," Journal of the British Interplanetary Society, Vol 17, pp 314-316, 1960.

35. Hatfield, W. D., "Activated Sludge," The Encyclopedia of Chemistry, p 15, Reinhold Publishing Corporation, New York, 1957.

36. Hayami, H., Shino, K., "Nutritional Studies on Decolourized Chlorella. Growth Experiment on Rats and the Digestibility of a Diet Containing 19% of Decolourized Chlorella," Annual Report of the National Institute of Nutrition (Tokyo, Japan), pp 56-58, 1958. Cited in Gini et al, 1960. Original reference not seen.

37. Hermann, E. R., "Reactor Design Criteria for Pond Stabilization of Wastewaters," Lecture Presented at Rice University, Houston, Texas, 1961.

38. Hermann, E. R., Gloyna, E. F., "Waste Stabilization Ponds," Sewage and Industrial Wastes, Vol 30, pp 511-538, 646-651, 963-975, 1958.

39. Heukelekian, H., Balmat, J. L., "Chemical Composition of the Particulate Fractions of Domestic Sewage," Sewage and Industrial Wastes, Vol 31, pp 413-423, 1959.

40. Hock, R. J., "The Potential Application of Hibernation to Space Travel," Aerospace Medicine. Vol 31, pp 485-489, 1960.

41. Hughes, E. O., Gorham, P. R., Zehnder, A., "Toxicity of a Unicellular Culture of Microcystic aeruginosa," Canadian Journal of Microbiology, Vol 4, pp 225-236, 1958.

42. Hundley, J. M., Ing, R. B., Krauss, R.W., "Algae as a Source of Lysine and Threonine in Supplementing Wheat and Bread Diets," Science, Vol 124, pp 536-537, 1956. Cited in Gini et al, 1960. Original reference not seen.

43. Huntner, S. H., Provasoli, L., Filfus, J., "Nutrition of Some Phagotrophic Fresh-Water Chrysamonads," Am. N.Y. Acad. Sci., Vol 56, pp 852-862, 1953.

44. Ingram. W. T., Ed., Engineering Biotechnology of Handling Wastes Resulting from a Closed Ecological System, AFOSR Report No.TR58-148 ASTIA Document AD162 277, December, 1958.

45. Ingram, W. M., Prescott, G. W., "Toxic Fresh-Water Algae," The American Midland Naturalist, Vol 52, pp 75-87, 1954.

46. Kountz, R. R., Forney, C., Jr., "Metabolic Energy Balances in a Total Activated Sludge System," Sewage and Industrial Wastes, Vol 31, pp 819-826, 1959.

47. Lackey, J. B., Hendrickson, E. R., "Biochemical Bases of Anaerobic Digestion," Biological Treatment of Sewage and Industrial Wastes, Vol II, Anaerobic Digestion and Solids - Liquid Separation, pp 9-25, Reinhold Publishing Corporation, New York, 1958.

48. Lavery, J., Tischer, R. G., "Food from Algae. A Review of the Literature," Quartermaster Food and Container Institute for the Armed Forces Library Bulletin No 1, Chicago, 1958.

49. Lorant, M., "Will Tilapia be Space Travellers' Food?," Food Manufacture, December, pp 479, 481, 1959.

50. Lubitz., J. A., "The Protein Quality, Digestibility and Composition of Chlorella 71105," Contract No. AF33(616)-7373, Project No. 61(7-6373), Task No. 63124, WADC Technical Report, Wright Air Development Center, Wright-Patterson Air Force Base, Ohio, 1961, (Report No. not assigned as yet)

51. Jorgensen, J., Convit, J., "Cultivation of Complexes of Algae with other Fresh-Water Microorganisms in the Tropics," Algal Culture from Laboratory to Pilot Plant, pp 190-196, Carnegie Institution of Washington Publication 600, 1953.

52. Malkin, V., "Astronautics and the Problem of Food," AID Report 61-41, Soviet Literature on Life Support Systems, Project PTT 541502, Work Assignment 22, Report 1, 24 March, p 10, 1961.

53. McDowell, M. E., Powell, R. C., Nevels, E. M., Sellars, J. H., Witt, N. F., "Algae Feeding in Humans: Acceptability, Digestibility and Toxicity," Fed. Proc., Vol 19, p 319, 1960.

54. McKinney, R. E., Conway, R. A., "Chemical Oxidation in Biological Waste Treatment," Sewage and Industrial Wastes," Vol 29, pp 1097-1106, 1957.

55. Meffert, M. E., "Algal Culture in Sewage," Transactions of the International Conference on the Use of Solar Energy - the Scientific Basis, Vol IV, Chap 8, The University of Arizona Press, Tuscon, Arizona, 1955.

56. Milner, H. W., "Possibilities in Photosynthetic Methods for Production of Oils and Proteins," Journal of the Amer. Oil. Chemists' Society, pp 363-367, August, 1951.

57. Morimura, Y., Tamiya, N., "Preliminary Experiments in the Use of Chlorella as Human Food," Food Technology, Vol 8, pp 179-182, 1954.

58. Myers, J., "Study of a Photosynthetic Gas Exchanger as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin, Part I," Report to the Air Research and Development Command, Air University School of Aviation Medicine, USAF, Randolph Air Force Base, Texas, 1955.

59. Myers, J., "Study of a Photosynthetic Gas Exchanger as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin, Part II," Report to the Air Research and Development Command, Air University School of Aviation Medicine, USAF, Randolph Air Force Base, Texas, 1957.

60. Myers, J. E., Brown, A. H., "Gas Regeneration and Food Production in a Closed Ecological System," National Academy of Sciences - National Research Council Publication 893, Washington, D. C., 1961.

61. Nakamura, H., "A Study of Wolffia as a New Food," Reports from the Microalgae Research Institute of Japan, Vol 2, pp 13-19, 1961.

62. Nakamura, H., Yamada, K., "Evaluation of the Nutritive Value of Unicellular Green Algae," Reports from the Microalgae Research Institute of Japan, Vol 1, pp 7-13, 1960.

63. Ney, L. F., Gas Exchange by Plants of the Duckweed Family, ONR Contract No. NOnr-2787(00), Stanford Research Institute, Menlo Park, California, 1960.

64. Nickell, L. G., Tulecke, W., "Submerged Growth of Cells of Higher Plants," Journal of Biochemical and Microbiological Technology and Engineering, Vol II, pp 287-297, 1960.

65. Oswald, W. J., "Metropolitan Wastes and Algal Nutrition," Transactions of the Seminar on Algae and Metropolitan Wastes, pp 88-95, USDHEW, Robert A. Taft Sanitary Center, Cinn. Ohio, 1960.

66. Oswald, W. J., "Light Conversion Efficiency of Algae Grown in Sewage," Journal of the Sanitary Engineering Division, Proceedings of the American Society of Civil Engineers, July, pp 71-95, 1960a.

67. Oswald, W. J., Gotaas, H. B., Golueke, C. G., Kellen, W. R., "Algae and Waste Treatment," Sewage and Industrial Wastes, Vol 29, pp 437-457, 1957.

68. Pierson, E. A., Crafts, J. H., Channin, G., Garher, W. F., Henderson, W. L., Kennedy, R. P., McKee, J. E., "Biocatalytic Additives in Sludge Digestion," Sewage and Industrial Wastes, Vol 29, pp 1066-1074, 1957.

69. Pipes, W. O., "Waste-Recovery Processes for a Closed Ecological System," National Academy of Sciences - National Research Council, Washington, D.C., 1961.

70. Pratt, R. F., Fong, J., "Studies on Chlorella vulgaris. II. Further Evidence that Chlorella Cells form a Growth - Inhibiting Substance," Amer. Journal Bot., Vol 27, pp 431-436, 1940.

71. Prosky, L., Karinen, J. F., "Nutritive Value of Algae with the Use of Weanling Rats," Abstract 236, Abstracts - Fifth International Congress on Nutrition, p 51, Washington, D. C., Sept. 1-7, 1960.

72. Provasoli, L., Pintner, I. J., "Ecological Implications of in vivo Nutritional Requirements of Algal Flagellates," Ann. N.Y. Acad. Sci., Vol 56, pp 839-851, 1953.

73. Rich, L. G., Ingram, W. M., Berger, B. B., "The Use of Vegetable Cultures as the Photosynthetic Component of Isolated Ecological Cycles for Space Travel," Advances in the Astronautical Sciences, Vol 6, pp 369-379, 1960.

74. Rose, S. M., "A Feedback Mechanism of Growth Control in Tadpoles," Ecology, Vol 41, pp 188-199, 1960.

75. Rose, W. C., Physiol. Rev., Vol 18, p 109, 1938. Cited in White et al, p 505, 1954. Original reference not seen.

76. Rudolfs, W., "Phosphates in Sewage and Sludge Treatment.I. Quantities of Phosphates," Sewage Works Journal, Vol 19, pp 1, 43, 1947.

77. Slobodkin, L. B., "Energetics in Daphnia pulex Populations," Ecology, Vol 40, pp 232-243, 1959.

78. Spector, W. S., Ed., Handbook of Biological Data, W. B. Saunders Co., Philadelphia, 1956.

79. Symons, J. M., McKinney, R. E., "The Biochemistry of Nitrogen in the Synthesis of Activated Sludge," Sewage and Industrial Wastes, Vol 30, pp 874-890, 1958.

80. Tamiya, H., Chemical Composition and Applicability as Food and Feed of Mass-Cultured Unicellular Algae, Final Report No. 1 ON Contract NODA 92-557-FEC-33129, U.S. Army Research and Development Group (9852) (Far East), 1961.

81. Taylor, A. A., "Present Capabilities and Future Needs for Space Feeding," Food Technology, Vol 12, pp 442-444, 1958.

82. Thomas, W. H., Krauss, R.W., "Nitrogen Metabolism in Scenedesmus as Affected by Environmental Changes," Plant Physiology, Vol 30, pp 113-122, 1955.

83. Thompson, G. V. E., "Sanitation in Space Means Recycling," Engineering, October, pp 552-553, 1960a.

84. Thompson, G. V. E., "Hydroponics Will Feed Men on the Moon," Engineering, August, p 294, 1960b.

85. Vinberg, G. G. (translated by G. Belkov), Toxic Phytoplankton, National Research Council of Canada Technical Translation TT-549, Ottawa, Canada, 1955.

86. Welch, B. E., "Space Logistics I. Food, Water, Waste," Lectures in Aerospace Medicine Conducted at the School of Aviation Medicine, Section 14, USAF Aerospace Medical Center (ATC), Brooks Air Force Base, Texas, 1960.

87. Whisenhunt, G. G., Jr., "Living in Space," Industrial Research, June-July, pp 19-23, 1961.

88. White, A., Handler, P., Smith, E. L., Stetten, D., Principles of Biochemistry, McGraw-Hill Book Co., Inc., New York, 1954.

89. Witkin, R., "Battery with Bacteria Catalyst Could Use Sea Matter as Fuel," The New York Times, Vol. CXI, pp 1, 20, October 25, 1961.

90. Zimmerman Process Division, Sterling Drug Inc., "Low Temperature Combustion of Sewage Sludge by the Zimmerman Process," New York, New York, 18 pp, 1960.

91. Zuraw, E. A., Christiansen, G. S., Kippax, D. L., Benoit, R. J., Richards, N. L., Huppert, I. N., Leone, D. L., Gaucher, T. A., Trainor, F. R., Noe, F. F., "Photosynthetic Gas Exchange in the Closed Ecosystem for Space," NASA Contract No. NASW-95, General Dynamics Corporation, Electric Boat Division, Groton, Connecticut, 1960.

APPENDIX B-1

EQUIPMENT SPECIFICATIONS FOR THE PGE SYSTEM

Gas Exchanger (1)

Size, 72" height x 25" diameter. Material, 304 stainless steel. Cylindrical tank (containing a lighting surface area of 10.2 sq. ft) including gas and cooling water manifolds. EB drawing numbers H-3094, H-3095, and H-3096. Fabricated at EBDivision.

Lamps (5)

Lamp size, 5/8" OD x 9" long. GE Quartzline Hi-Intensity Incandescent Lamp (Tungsten filaments). Volts 277, amps 7.3, watts 1500. Manufactured by General Electric Company.

Pump (nutrient feed) (1)

Model 1106; Type 0372; 3.2 gph; stainless steel water encl.; 1/4 hp, 115 V, single phase, 60 cycle, TE motor. BIF Industries, Inc.

Nutrient Storage Tanks (2)

Stainless steel, 55 gal. drums

Pump (Gas) (1)

Size 22, Type XA, carbon steel, rotary, positive displacement, gas tight seals, equipped with base plate, V-belt drive and guard. 3/4 hp, 1725 rpm, 3 phase, 60 cycle, 220/440 V, drip proof induction motor. Leiman Bros.

Surge Tank (1)

80 gallon pressure tank, SST, 48" long x 24" diameter. Rated for 400 psi. A.C. Tank Co.

APPENDIX B-2a

INSTRUMENTATION FLOW SHEET DESCRIPTION

General

Drawing 3063 (Appendix B-2b) shows the instrumentation of the PGE system. All items are labeled according to the code shown on Drawing 3065 (Material List). The electrical schematic is shown on Drawing 3064. Individual instrument specifications are given in Appendix B-2c.

Level Control

The flow of a premixed nutrient solution into the PGE was governed by a metering pump. When the liquid level L-1 was reached, relay R1 locked up (in LRB-1) through R2, opening LCV-5A through PrR5-a. Draining of algae ceased when the liquid level fell below L2 (R1 drops out, R2 drops out, lighting L2 level lamp SL-6). A 1/8-in. spacing between probes L1 and L2 was used for differential gap control.

Lamp Power Control

The five lamps were operated at 208 volts, 60 cycles, through permissive power relay PrR1-2. This relay was locked in by the following:

Algae Temperature (RTX-1 and TX-1)

Level (L3 and R3)

Cooling Water (PX-5)

Circulating Gas (DPX-4)

The lamp voltages were hand-set by Variacs (PrC-1 thru 5).

Algae Temperature

Simple on-off control of cooling water flow was used. Temperature control switch TCX-1 actuated temperature control valve TCV-1. Actual algae temperature was read on TI-1.

System Pressure

The vent-gas passed through an absolute back pressure valve, PCV-vent, set at approximately 15 psia. Therefore, at system pressures below 15 psia, the back pressure valve was closed. Gas was allowed to vent from the system only when the system pressure exceeded 15 psia.

Inlet Gas Flow

CO_2 and N_2 inlet flows were manually set and automatically controlled by F1CV-CO_2 and F1CV-N_2. Inlet flow valves were periodically checked by temporarily by-passing through calibrated wet-test meters (not shown).

Low gas supply pressures actuated an alarm thru PX-CO_2 and PX-N_2.

Power Supply

System power, plus energizing of the 1) Nutrient Pump, 2) Agitator, 3) Circulating Pump, and 4) Lamp Voltage, was accomplished through use of locking relays. On power failure or shutdown, relays had to be reset manually.

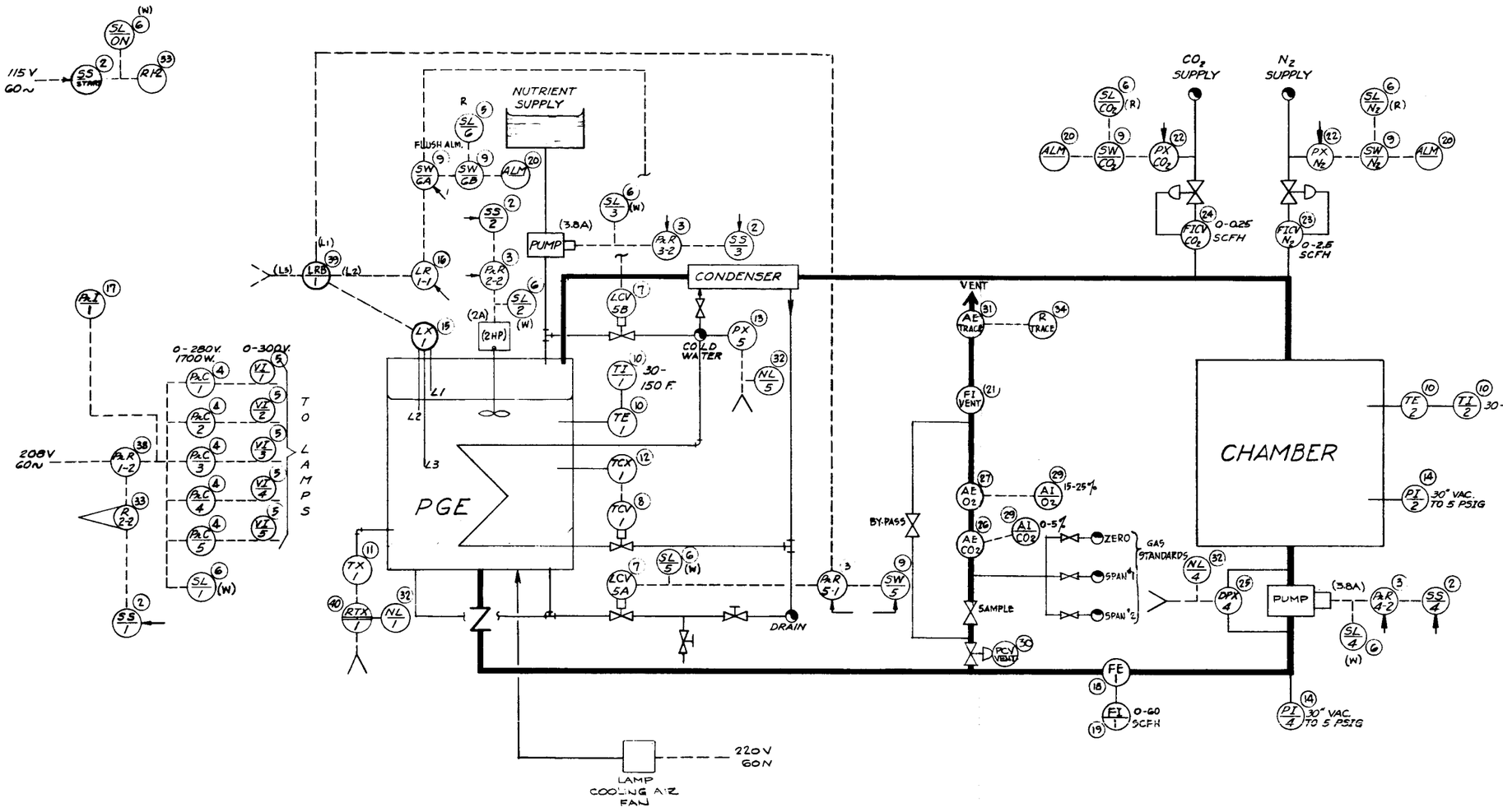
Page intentionally left blank

APPENDIX B-2b

INSTRUMENTATION DRAWINGS

Page intentionally left blank

SYM	CHG. ORDER	REVISIONS	DATE	BY	APP.
A		DRAWING NUMBER WAS 3108	4-6-61	FWM	



PROCESS MOUNTED INSTRUMENT

PANEL MOUNTED INSTRUMENT

115 VOLT POWER TO LAMP PERMISSIVE RELAY R2-2

SIGNALS LOCKING R2-2

115 V HELD IN BY "START" RELAY R1-2

ITEM NO	REQD	PART NO	DESCRIPTION	MATL	MATL SPEC	UNIT WT

LIST OF MATERIAL

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES
TOLERANCES ON
FRACTIONS DECIMALS ANGLES
MATERIAL
HEAT TREATMENT
FINAL PROTECTIVE FINISH

GENERAL DYNAMICS CORPORATION
ELECTRIC BOAT DIVISION, GROTON, CONN.
RESEARCH & DEVELOPMENT DEPARTMENT

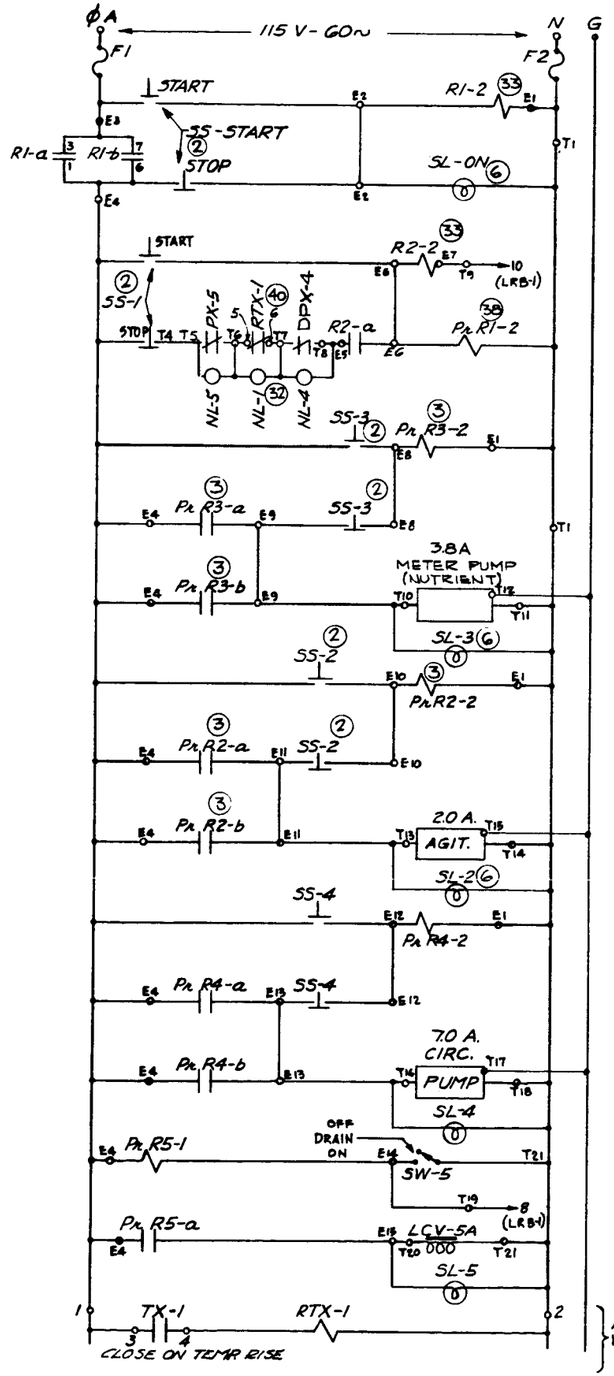
	BY	DATE
DRAWN	EWP	1-13-61
CHECKED	RJN	1/31/61
EXAMINED		
APPROVED		
SCALE		

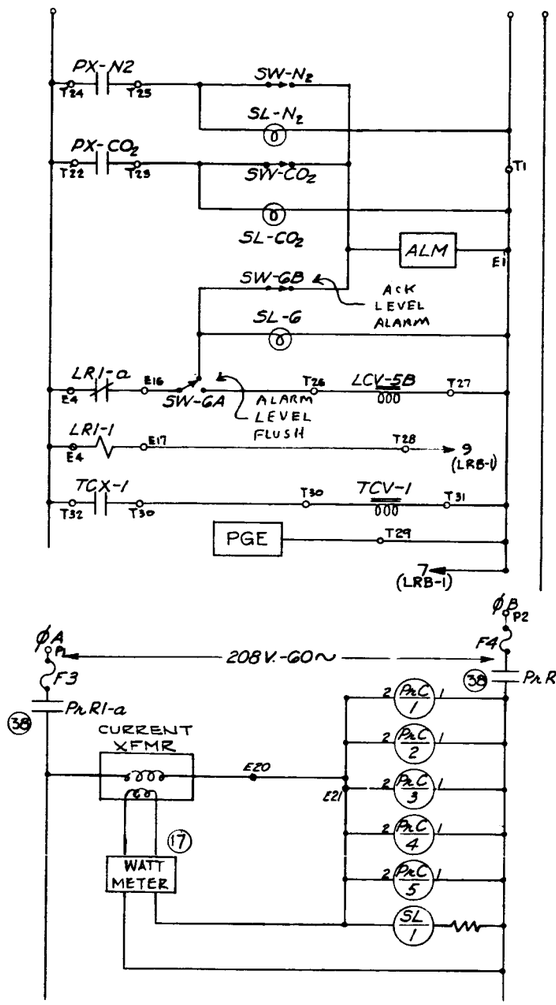
DEVICE NO
DWG TITLE
NASA CONTRACT
NASS-GIG — PHASE II
INSTRUMENTATION FLOW CHART

DWG SIZE D
DWG NO 3063
SHEET NO OF

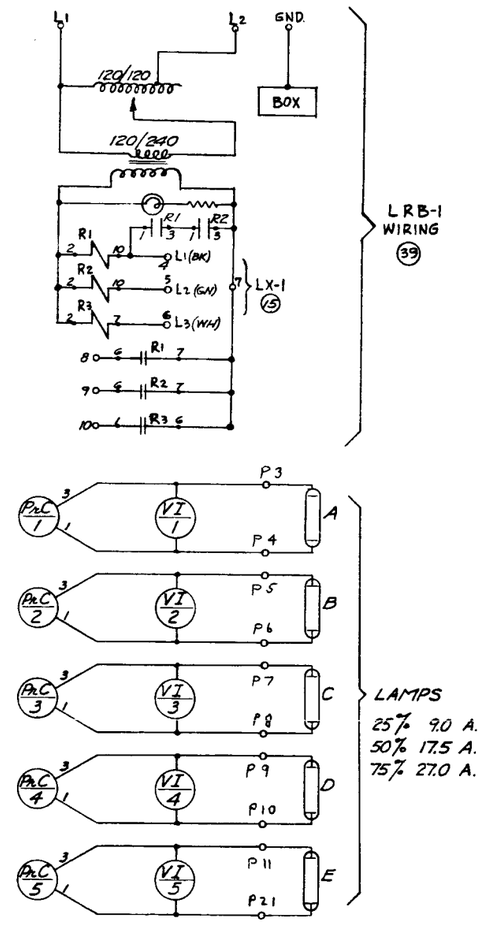
Page intentionally left blank

SYM	CHG. ORDER	REVISIONS	DATE	BY	APP.
A		DRAWING NUMBER WAS 3109	4-6-61	FWW	





T - EXTERNAL CONNECTIONS
E - PRE-WIRED RELAY BOX



ITEM NO	REQD	PART NO	DESCRIPTION	MATL	MATL SPEC	UNIT WT

LIST OF MATERIAL

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES
TOLERANCES ON
FRACTIONS DECIMALS ANGLES
MATERIAL
HEAT TREATMENT
FINAL PROTECTIVE FINISH

GENERAL DYNAMICS CORPORATION
ELECTRIC BOAT DIVISION, GROTON, CONN.
RESEARCH & DEVELOPMENT DEPARTMENT

	BY	DATE
DRAWN	EWP	11/16/61
CHECKED	RJN	11/17/61
EXAMINED		
APPROVED		
SCALE		

DEVICE NO
DWG TITLE
NASA CONTRACT
NAS5-616 —— PHASE II
INSTR. CONTROL SCHEMATIC

DWG SIZE C
DWG NO 3064
SHEET NO OF

Page intentionally left blank

SPEC. SHEET	SYMBOL	NUMBER	ITEM
1	—	—	CABINET
2	SS	1, 2, 3, 4, START	1 SET OF PUSH BUTTONS N.O. & N.C.
3	Pr R	2-2, 3-2, 4-2, 5-1	DPDT POWER RELAY 115V. COIL
4	Pr C	1, 2, 3, 4, 5	230 V. VARIAC
5	VI	1, 2, 3, 4, 5	VOLTMETER
6	SL	1, 2, 3, 4, 5 N_2, CO_2, G, ON	SIGNAL LAMP
7	LCV	5A, 5B	2 WAY SOL. VALVES 115 V. E/O
8	TCV	1	SAME AS LCV-5A
9	SW	5, GA, GB, N_2, CO_2	DPDT TOGGLE
10	TE, TI	1, 2	TEMP. INDICATOR
11	TX	1	TEMP. SWITCH
12	TCX	1	SAME AS TX-1
13	PX	5	PRESSURE SWITCH
14	PI	4, 2	PRESSURE GAGE
15	LX	1	SPECIAL LEVEL PROBE
16	LR	1-1	DPDT RELAY
17	PrI	1	WATT METER
18	FE	1	SPECIAL ORIFICE

SYM	CHG. ORDER	REVISIONS	DATE	BY	APP.
A		DRAWING NUMBER WAS 3110	4-5-61	FDH	

SPEC SHEET	SYMBOL	NUMBER	ITEM
19	FI	1	PRESSURE GAGE
20	ALM	—	ALARM UNIT
21	FI	VENT	WET METER
22	Px	N_2, CO_2	HIGH PRESSURE SWITCH
23	FICV	N_2	FLOW CONTROLLER
24	FICV	CO_2	FLOW CONTROLLER
25	DPX	4	DIFFERENTIAL PRESSURE SW.
26	AE	CO_2	INFRA-RED ANALYZER
27	AE	O_2	PARAMAGNETIC ANALYZER
28			
29	AI	CO_2, O_2	VOLT-METER
30	PCV	VENT	BACK PRESSURE CONTROLLER
31	AE	TR	CHROMATOGRAPHIC ANALYZER
32	NL	1, 4, 5	NEON LAMP
33	R	1-2, 2-2	3PDT 115V RELAY
34	R	TR	TRACE ANALYZER RECORDER
35	F	1, 2	FUSES - 115V 15A
36	F	3, 4	FUSES - 208V 30A
37			
38	Pr R	1-2	DPDT POWER RELAY 208V COIL
39	LRB	1	LEVEL RELAY BOX
40	RTX-1	1	TEMP. SWITCH-REV. RELAY BOX

ITEM NO	REQD	PART NO	DESCRIPTION	MATL	MATL SPEC	UNIT WT

LIST OF MATERIAL

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES TOLERANCES ON

FRACTIONS DECIMALS ANGLES

MATERIAL

HEAT TREATMENT

FINAL PROTECTIVE FINISH

GENERAL DYNAMICS CORPORATION
ELECTRIC BOAT DIVISION, GROTON, CONN.
RESEARCH & DEVELOPMENT DEPARTMENT

	BY	DATE
DRAWN	EWP	1/16/61
CHECKED	RJN	1/17/61
EXAMINED		
APPROVED		
SCALE		

DEVICE NO
DWG TITLE
NASA CONTRACT
NAS5-616 PHASE II
MATERIAL LIST

DWG SIZE B

DWG NO A 3065

SHEET NO OF

Page intentionally left blank

APPENDIX B-2c

INSTRUMENTATION SPECIFICATIONS

Power Relays (4)

Model PR11AY, coil 115V, contacts 25A, Potter and Brumfield

Level Control Valves (2)

Coil 115V, voltage 115V, DOLE

Temperature Control Valve (1)

Coil 115V, voltage 115V, DOLE

Temperature Indicator (2)

Model 907S, range +30 to +150°F, U.S. Gauge

Temperature Switch (1)

Model 17001-12, contacts 10A, 115V, 5A, 220V, Range - 100 to +400°F Fenwal

Temperature Control Switch (1)

Model 17001-12, Range -100 to +400°F, contacts 10A, 115V, 5A, 220V, Fenwal

Pressure Switch (3)

Model C-9612-0-WA, Range 15 to 200 psig, coil, 115V, contacts 10A Barksdale

Pressure Indicator (2)

Model 505SR, Range 30" vacuum to 5 psig; retard to 30 psig, U.S. Gauge

Flow Indicator (1)

Model 633 SV, Range 0-36 oz/in.^{2}, U.S. Gauge

Flow Indicating Control Valve (1)

Model 63BD4A, Range 0-2.5 SCFH, Moore Products

Flow Indicating Control Valve (1)

Range 0-0.25 SCFH, Moore Products

Differential Pressure Switch (1)

Model 427-E-10L, Range 0.2 to 15 psid, Barksdale

Back Pressure Control Valve (1)

Model 43R, Range 0-20 psia, Moore Products

Level Relay Box (1)

Symbol LRB-1, 115 V, Electric Boat Division

Transformers, variable (5)

Superior type 226. Input 0-220V, single phase, 60 cycle; output 0-290V, single phase, 60 cycle, 1.7 KVA maximum. Superior Electric Company

Voltmeters (5)

Triplett model 237-PL. Range 0-300V, single phase, 60 cycle, Newark Electric Company

Vapor Fractometer (1)

Model 154C, used with "I" and "J" columns in parallel adapter. Printing integrator unit. Leeds and Northrup model 69800 recorder. Perkin-Elmer Company

Infrared Analyzer (1)

L/B model 15A amplifier, model 63130 L/B analyzer No. 61967. Beckman Instrument Company

Recorder (1)

24 point, range 0-5 MV, model 6702, type 1D PAK. Daystrom Weston, Inc.

Test Meter (1)

Wet test meter, 0.25 cubic feet gas per revolution. Precision Scientific Company

Paramagnetic Analyzer (1)

Model F3N3, 1A3A oxygen analyzer. Ranges 0-25, 15-25%. 115V, 60 cycles, 0-25 MV output. Beckman Instrument Company

APPENDIX C-1

SUMMARY OF DATA OF FACTORIAL EXPERIMENTS

	Agitation Rate (strokes/min)					
	45			90		
	Light Intensity (KW)			Light Intensity (KW)		
	2.40	5.85	7.50	2.40	5.85	7.50
Algae dry weight (mg/L)	296 322	445 686	618 670	326 360	594 563	805 589
Algae PCV (%Vol/Vol)	0.13 0.11	0.19 0.30	0.24 0.28	0.16 0.15	0.26 0.24	0.33 0.30
O_2 Produced (SCFH)	0.067 0.100	0.081 0.147	0.112 0.154	0.078 0.077	0.113 0.140	0.138 0.124
CO_2 Consumed (SCFH)	0.048 0.067	0.075 0.104	0.081 0.111	0.052 0.055	0.089 0.119	0.112 0.092
AQ (Rotameters)	0.830 0.718	0.963 0.791	0.721 0.740	0.685 0.731	0.794 0.862	0.825 0.808
Gas Balance (Rotameters %)	91.1 71.0	109.5 79.5	66.3 86.4	72.8 80.9	71.8 78.9	84.2 74.6
Carbon Balance* (%)	94.20 119.30	102.28 88.45	86.00 97.92	97.34 91.80	116.80 97.78	91.35 96.64
Chlorophyll (%)	3.43 4.25	3.61 2.45	2.32 3.66	2.73 3.48	3.70 3.85	1.22 2.45
Nitrogen Balance (%)	106.2 92.0	109.3 93.0	- -	101.1 98.3	100.1 96.1	- -
Ash (%)	7.05 5.74* 6.46 5.72*	7.25 6.58* 3.18 2.72*	6.99 6.02* 7.33 7.16*	5.98 5.41* 4.02 3.26*	7.93 6.90* 7.02 6.31*	6.45 5.33* 6.99 5.18*
N_2 Consumed (mg/10 ml)	0.264 0.446	0.393 0.772	0.524 -	0.305 0.424	0.624 0.730	- -
Carbon (%)*	51.30 50.72	49.92 53.46	47.66 50.52	49.27 52.08	50.62 50.64	47.71 48.14
Nitrogen (%)	11.05 10.79* 11.23 10.83*	10.85 10.80* 11.72 11.61*	9.91 9.74* 11.20 10.75*	9.69 9.38* 11.32 11.15*	10.97 10.90* 11.33 10.69*	10.01 9.95* 9.89 9.51*
Hydrogen (%)*	7.12 7.08	7.24 7.34	7.22 7.22	7.62 7.11	7.02 7.06	7.05 7.10

*Analysis by the Galbraith Laboratories Inc., Knoxville 21, Tenn.

APPENDIX C-2

TABULATION OF THE ANALYSIS OF VARIANCE

Analysis of Variance Effect	Degrees of Freedom	RESPONSE								
		O_2	Total Gas Flow	CO_2	Elemental Analysis H	Elemental Analysis N	Elemental Analysis C	Ash	Chloro-phyll	Dry Wt.
(A) Light	2	*		*		**	*		●	●
Linear	(1)	(*)		(*)			(*)		(*)	(*)
Quadratic	(1)									
(B) Agitation	1									
(AB) Interaction	2									
Error	6									
Magnitude Index										
(A) Light+		3,2,1	3,1,2	3,2,1	1,2,3	2,1,3	2,1,3	3,2,1	1,2,3	3,2,1
(B) Agitation++		2,1	2,1	2,1	1,2	1,2	1,2	2,1	1,2	2,1
Components of Variance										
(A) Light+										
1 vs. 2		*		*						*
2 vs. 3						**	*		●	
3 vs. 1		*		*			*		*	*
(B) Agitation++										
1 vs. 2										

*Significant @ 95% Level
**Barely Significant

\+ 1 = 4.20 KW Power Input
2 = 5.85 KW " "
3 = 7.50 KW " "

++ 1 = 45 cycles/min
2 = 90 " "

APPENDIX C-3

CALCULATION OF THEORETICAL GAS EXCHANGE

SAMPLE CALCULATION

$$0.5(N_2H_4CO) + 5.04\ CO_2 + 3.64\ H_2O \longrightarrow C_{5.5}\ H_{9.29}\ O_{2.03}\ N + 6.1\ O_2$$

Mol. wt = 121.3 + 5.74 ash = 127.04 cell wt

O_2

6.1 moles x 22.4 ℓ/mole = 136.6 liters

$\frac{136.6}{127.04}$ = 1.075 ℓ/gm

$\frac{1.075\ \ell/gm}{28.32\ \ell/cu\ ft}$ = 0.038 cu ft/gm

CO_2

5.04 moles x 22.4 ℓ/mole = 112.8 liters

$\frac{112.8}{127.04}$ = 0.888 ℓ/gm

$\frac{0.888\ \ell/gm}{28.32\ \ell/cu\ ft}$ = 0.0313 cu ft/gm

APPENDIX C-4

CALCULATION OF MATERIAL BALANCES

CARBON BALANCE

Run #11

CO_2 input flow = 0.095 SCFH

CO_2 vent flow = 0.005 SCFH

Suspension flow = 4.9 liters/hour

Algal density = 0.563 grams dry algae/liter

% Carbon in algae = 50.64

$$\text{Carbon input} = 0.095\ CO_2 \times \frac{1\ \text{lb-mol}}{359} \times \frac{44\ \text{lb}\ CO_2}{1\ \text{lb-mol}\ CO_2} \times \frac{12\ \text{lb carbon}}{44\ \text{lb}\ CO_2}$$

$$= 0.003175\ \text{lb carbon}$$

$$\text{Carbon vent} = 0.005\ CO_2 \times \frac{1\ \text{lb-mol}}{359} \times \frac{44\ \text{lb}\ CO_2}{1\ \text{lb-mol}\ CO_2} \times \frac{12\ \text{lb carbon}}{44\ \text{lb}\ CO_2}$$

$$= 0.000167\ \text{lb carbon}$$

$$\text{Carbon (in suspension)} = 4.9\ \frac{\text{liters}}{\text{hr}} \times 0.563\ \frac{\text{grams dry algae}}{\text{liter}} \times \frac{1\ \text{lb}}{454\ \text{grams}} \times \frac{0.5064\ \text{lb carbon}}{1\ \text{lb dry algae}}$$

$$= 0.003080\ \text{lb carbon}$$

$$\text{Total carbon leaving system} = 0.003080 + 0.000167\ \text{lb}$$

$$= 0.003247\ \text{lb}$$

$$\text{Total carbon entering system} = 0.003175\ \text{lb}$$

$$\%\ \text{Carbon accountable} = \frac{0.003175}{0.003247} \times 100 = 97.8\%$$

NITROGEN BALANCE

Run #5

mg/liter of nitrogen in nutrient media = 97.12
mg/liter of nitrogen in supernatant = 66.60
Culture density in mg dry weight/liter = 326.0
% nitrogen in dry algae = 9.69

Note: Nutrient flow rate = supernatant flow rate

Nitrogen inlet flow = 97.12 mg/liter

$$\text{Nitrogen exit flow} = 66.60 \frac{\text{mg}}{\text{liter}} + \frac{326 \text{ mg dry algae}}{\text{liter}} \times \frac{0.0969 \text{mg } N_2}{1 \text{ mg dry algae}}$$

Nitrogen exit flow = 98.2 mg/liter

$$\% \text{ Nitrogen accountable} = \frac{98.2}{97.1} \times 100 = 101.1\%$$

TOTAL GAS FLOW BALANCE

Run #8

CO_2 inlet flow + N inlet flow = total inlet flow
Total inlet flow = 0.286 SCFH
Total exit flow = 0.314 SCFH

$$\% \text{ total gas flow accountable} = \frac{.286}{.314} \times 100 = 91.08\%$$

APPENDIX C-5

LIGHT MEASUREMENTS WITHIN THE PGE

REDUCTION OF THE DATA OF MYERS AND GRAHAM TO WORKING EQUATIONS

The data, as presented, consisted of a series of curves of OD vs depth with algal cell concentration the parameter. To determine optical density as a function of both culture depth and algal density, this procedure was followed:

1. Optical density was plotted vs depth (at fixed algal densities) in various ways (e.g. log-log, semi-log, linear, etc.) until linear curves were obtained. This required separating the data, thereby establishing several series of linear plots.

2. The slopes and intercepts of the linear curves were evaluated.

3. The slopes and intercepts obtained from step (2) were plotted as a function of algal cell concentration to obtain linear curves.

4. The slopes and intercepts of these linear curves were evaluated.

5. The slopes and intercepts from step (4) were substituted into the general form of the equations determined in step (1). For example, in step (1) it was found that OD varies linearly as the log X, where X is culture depth. The general equation is then $OD = m \log X + b$, and m and b, the slope and intercept, were evaluated as a function of algal cell concentration in step (4).

For the data of Myers and Graham, the data was first separated into two situations: measurements taken at depths less than 75 mm, and those at depths greater than 75 mm. The data below 75 mm were taken as being linear.

Above 75 mm, the optical density was found to vary linearly as the log of depth, and the slopes and intercepts obtained from these linear plots were found to yield straight lines when plotted against algal density on log-log paper. These curves are shown in Figures A-1 and A-2. As shown in Figure A-2, the last set of points fall somewhat off the straight lines, necessitating the formation of an-

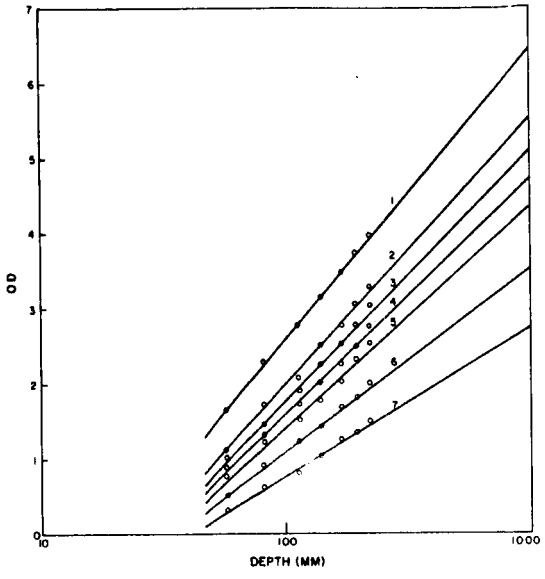


FIGURE A-1 DATA OF MYERS AND GRAHAM LINEARIZED BY PLOTTING DEPTH ON A LOG SCALE

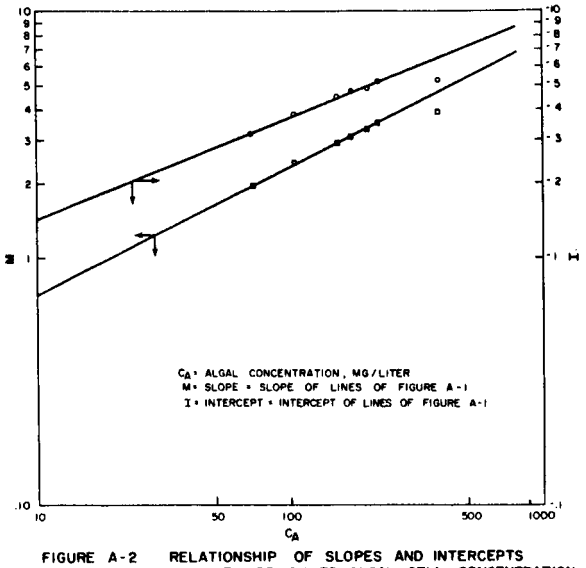


FIGURE A-2 RELATIONSHIP OF SLOPES AND INTERCEPTS FROM FIGURE A-1 TO ALGAL CELL CONCENTRATION

other situation for depths greater than 75 mm and for concentrations above 230 mg/liter. As a result the following equations were determined:

Situation I

$X > 75$ mm

$C_A < 230$ mg/liter

$$OD = 0.217\ C_A^{0.515} \log X - 0.561\ C_A^{0.409} + (10^{-5})\ X^2 \log C_A$$

Situation II

$X > 75$ mm

$C_A > 230$ mg/liter

$$OD = 1.43\ C_A^{0.168} \log X - 5.2$$

The same procedure was followed for the data at depths less than 75 mm, and the corresponding equations were:

Situation III

$X < 75$ mm

$C_A < 230$ mg/liter

$$OD = \frac{X}{75}\ (0.00585\ C_A + 0.12)$$

Situation IV

$X < 75$ mm

$C_A > 230$ mg/liter

$$OD = \frac{X}{75} (0.00372\ C_A + 0.64)$$

Data fits for the derived equations are shown in Figures A-3, A-4, A-5, and A-6. The points noted on Figures A-5 and A-6 as EB data were obtained by diluting a 1.60% (by volume) algal suspension to suspension densities of 0.16% and 0.027% (by volume). The optical densities of the two diluted suspensions were measured with a Fisher Electrophotometer, using a 0.9 neutral filter, at 10, 20, and 40 mm depths.

DERIVATION OF A GENERAL LIGHT INTENSITY FUNCTION

The distribution of light within the algal suspension, as derived from the data of Myers and Graham, is as follows:

For an algal depth less than 75 mm; $C_A > 230$ mg/liter

$$(1)\ OD = \frac{X}{75}\ 0.00372\ C_A + 0.64\ ,$$

and for a depth greater than 75 mm; $C_A > 230$ mg/liter

$$(2)\ OD = 1.43\ C_A^{0.168} \log X - 5.2$$

where:

X = algal depth (mm)
C_A = algal density (mg/liter)
OD = optical density

Since (3) $OD = \log I_o/I$

where: I_o = initial incident light intensity (foot-candles)
I = light intensity at position X (foot-candles)

the above equations may be rewritten as:

$$(4)\ \log \frac{I_o}{I} = \frac{X}{75} (0.00372\ C_A + 0.64) \text{and,}$$

$$(5)\ \log \frac{I_o}{I} = 1.43\ C_A^{0.168} \log X - 5.2$$

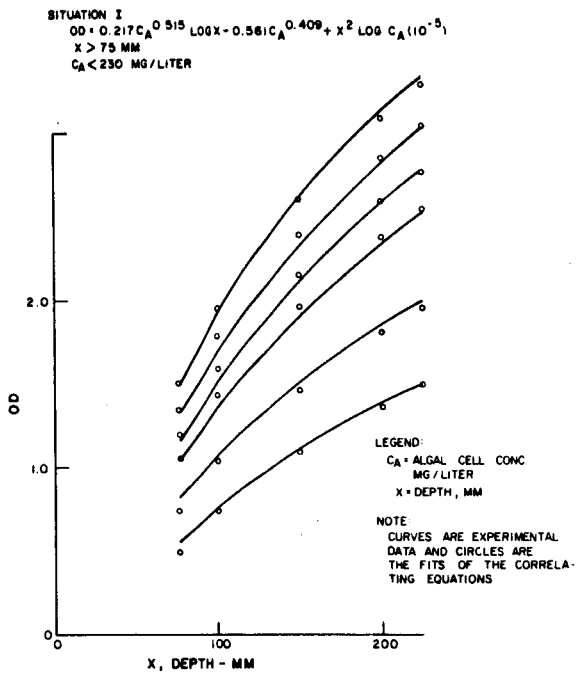


FIGURE A-3 CORRELATING EQUATION FOR DATA OF MYERS AND GRAHAM

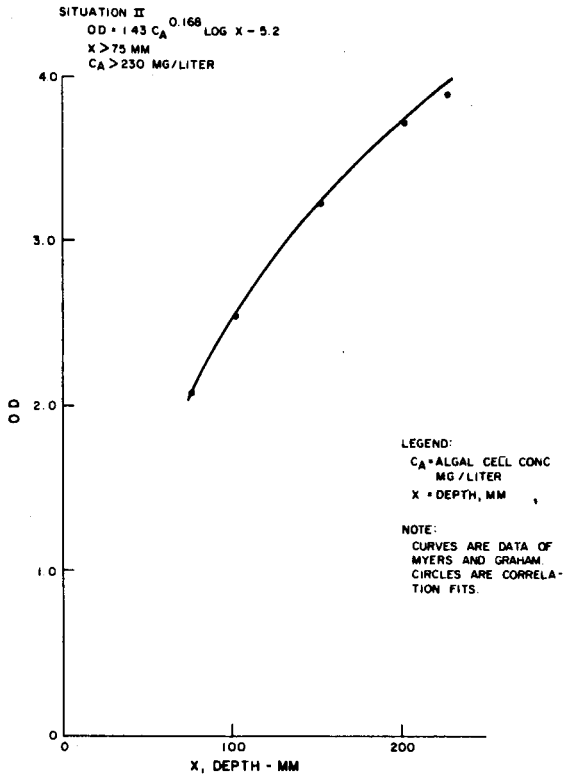


FIGURE A-4 CORRELATING EQUATION FOR DATA OF MYERS AND GRAHAM

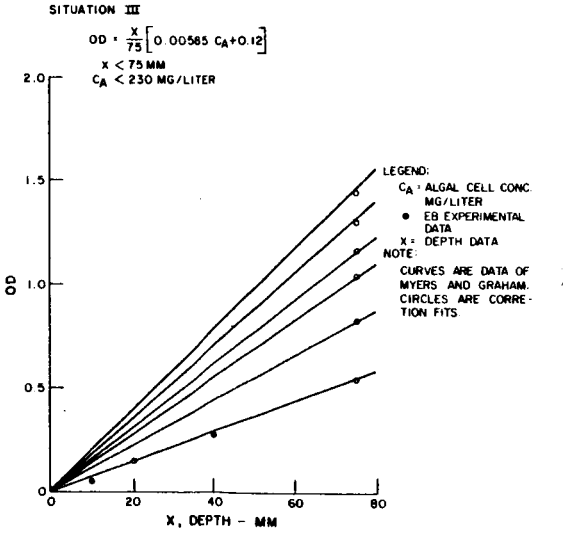


FIGURE A-5 CORRELATING EQUATION FOR DATA OF MYERS AND GRAHAM

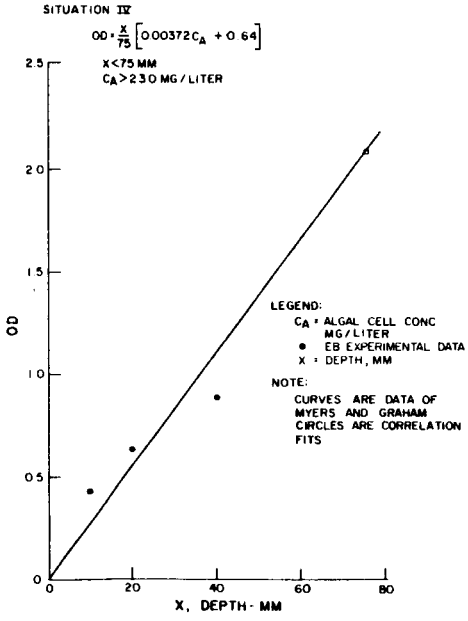


FIGURE A-6 CORRELATING EQUATION FOR DATA OF MYERS AND GRAHAM

The light intensity at the surface of the glass dome (I_o) has been represented by the expression:

$$(6)\quad I_o = a_o + a_1 y + a_2 y^2 + a_3 y^3 + a_4 y^4$$

where y = vertical distance from base of glass dome (inches) the values for the coefficients of equation (6) are given in Table 11.

Equations (4) and (5) may be rewritten in the following form:

$$(7)\quad I = I_o\, e^{-\left[\frac{X}{75}\,(0.00372\, C_A + 0.64)\right]} \qquad \text{and}$$

$$(8)\quad I = I_o\, e^{-\left[1.43\, C_A^{0.168}\, \log X - 5.2\right]}$$

Finally, by replacing I_o by equation (6), the general form obtained is:

For $X < 75$ mm; $C_A > 230$ mg/liter

$$(9)\quad I = (a_o + a_1 y + a_2 y^2 + a_3 y^3 + a_4 y^4)\, e^{-\left[\frac{X}{75}\,(0.00372\, C_A + 0.64)\right]}$$

and for $X > 75$ mm; $C_A > 230$ mg/liter

$$(10)\quad I = (a_o + a_1 y + a_2 y^2 + a_3 y^3 + a_4 y^4)\, e^{-\left[1.43\, C_A^{0.168} \log X - 5.2\right]}$$

Similarly, equations for algal cell concentration less than 230 mgs/liter may be derived starting with equations describing situations I and III.

APPENDIX D-1

CHLOROPHYLL EXTRACTION METHOD

A 75-100 mg sample of dried algae was extracted with a 1:1 mixture of ethyl ether-methanol at room temperature.

This extraction was performed in a 50-ml centrifuge tube by stirring the algae with solvent, centrifuging it, and removing the supernatant. The algae was then transferred from the tube, with solvent, to a small mortar and pestle. The excess solvent was removed, leaving the dry powder. This powder was ground very fine and then triturated with a little solvent; additional solvent was added and the mixture of powder and solvent was centrifuged. This process was repeated until the algae was pale green and the supernatant almost colorless. The collected supernatants were transferred to a 250-ml separatory funnel and a saturated NaCl solution was added to change the solubility. Two phases were formed: an aqueous methanol layer and an ether layer containing the pigment. The aqueous phase was removed, and the ether phase was washed once with water. The ether layer containing the chlorophyll was then transferred to a 100-ml volumetric flask and made to volume with ether. Before spectrophtometic analysis, the extract was centrifuged briefly in order to remove minute quantities of water.

APPENDIX D-2

PROCEDURE FOR DETERMINING THE EQUILIBRIUM DENSITY

1. At equilibrium, withdraw a 500-ml aliquot of algal culture from the PGE.

2. Place this suspension in the centrifuge and run for 20 minutes at 2,000 rpm. Discard the supernatant liquid.

3. Wash the recovered cells three times with de-ionized water.

4. After washing, add water to adjust the total volume to 50 ml. (This concentrates the cells to 10 times the original concentration.)

5. Place duplicate 3-ml samples of this suspension in calibrated hematocrit tubes and centrifuge the samples. Compute the average packed cell volume (PCV).

6. Place duplicate 20-ml samples of the concentrated suspension into previously dried and weighed aluminum dishes and dry in an oven at 105°C for 24 hours.

7. After drying, weigh the cooled samples and compute the dry weight (mg/ml).

APPENDIX D-3a

ELECTRIC BOAT ELEMENTAL ANALYSES OF ALGAE

Run	Moisture (%)	Ash (%)	Nitrogen (%)	Total Chlorophyll (%)	Chlorophyll-a (%)	Chlorophyll-b (%)	Nutrient Media Nitrogen (mg/liter)	Supernatant Nitrogen (mg/liter)
1	9.62 9.47	5.84 5.83	10.04 9.99	1.04 1.16	0.59 0.66	0.45 0.50	Not run	Not run
2	9.29 9.40	6.15 5.98	9.05 9.09	1.42 1.65	0.83 0.99	0.58 0.47	Not run	Not run
3	8.40 8.62	6.33 6.46	9.05 9.10	2.52 2.14 2.11	1.44 1.26 1.22	1.07 0.86 0.87	Not run	Not run
4	8.29 8.13	6.19 6.64	8.96 9.19	2.12 2.35 2.29	1.25 1.31 1.31	0.99 1.06 0.99	Not run	Not run
5	7.17 6.84	5.56 5.58	9.04 9.00	2.44 2.64	1.47 1.62	0.96 1.02	97.5 96.8	66.6 66.6
6	10.24 11.04	6.79 6.34	9.73 9.74	3.26 3.20	1.85 1.92	1.38 1.28	94.0 95.8	53.6 57.4
7	10.07 10.06	3.83 3.98 3.99	9.03 9.26	1.31 1.27	0.89 0.83	0.42 0.59	107.2 107.0 100.6	73.8 74.8 73.8
8	8.32 8.34	6.43 6.52	10.05 10.15	3.07 3.21	1.94 2.04	1.14 1.70	105.0 101.6 97.6	75.2 75.2
9	9.41 9.36	6.68 6.63	10.10 10.33 10.04 10.19	3.43 3.22	2.19 2.02	1.22 1.20	107.3 101.2 99.0 103.7	49.5 51.3
10	7.09 7.22	3.51 3.72 3.96	10.45 10.64	3.21 3.28	2.01 2.07	1.20 1.22	100.8 100.4 101.6	56.4 58.4
11	8.75 8.77	6.47 6.53 6.42	10.37 10.37 10.34 10.21	3.57 3.48	2.26 2.23	1.31 1.25	112.6 115.4 117.8 119.6	43.4 45.6 42.6
12	8.83 8.79	5.98 5.81	10.26 10.23	3.88 3.88	2.48 2.45	1.40 1.40	105.3 104.6	60.9 59.8
13	8.66 8.88	2.91 2.91	10.61 10.79	2.35 2.17	1.57 1.46	0.75 0.71	100.9 100.9 93.0	10.9 10.9
14	9.35 9.66	7.34 7.69	10.09 9.90	3.45 3.01	2.17 2.15	1.27 1.16	71.8 73.5	10.9 10.9
15	7.57 7.56	4.28 4.11	9.18 8.99	1.83	1.25	0.58	85.5 85.4	5.5 4.8

APPENDIX D-3b

GALBRAITH LABORATORIES ELEMENTAL ANALYSES OF ALGAE

Run	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Moisture (%)	Ash (%)
1	47.53 47.89	7.04 7.06	9.89 10.02	3.97 4.20	5.20 5.46
2	48.20 48.34	7.20 7.40	9.36 9.28	4.12 4.30	6.64 6.35
3	47.45 47.88	7.35 7.10	9.66 9.82	4.35 4.59	6.02 6.03
4	48.13 48.15	7.28 6.93	9.41 9.62	4.50 4.51	5.30 5.07
5	49.08 49.35	7.46 7.77	9.35 9.40	3.64 3.74	5.23 5.59
6	50.01 49.82	7.21 7.28	10.81 10.78	7.96 7.99	6.46 6.69
7	50.95 50.71	7.56 7.33	9.81 8.85	8.09 8.12	4.12 3.91
8	51.38 51.21	7.09 7.14	10.70 10.88	7.76 7.91	5.65 5.83
9	50.41 50.64	7.22 7.23	10.88 10.62	7.80 7.87	7.11 7.20
10	51.98 52.19	7.19 7.03	11.18 11.12	7.34 7.53	3.19 3.34
11	50.79 50.88	7.16 6.95	10.79 10.59	7.56 7.37	6.19 6.43
12	50.61 50.82	7.21 6.98	10.79 10.87	7.69 7.70	5.62 5.81
13	53.48 53.45	7.26 7.41	11.51 11.71	8.21 8.41	2.62 2.82
14	50.62 50.63	7.10 6.95	10.85 10.95	7.97 7.93	6.88 6.93
15	51.06 51.22	7.46 7.53	9.35 9.38	7.55 7.49	3.85 4.00

APPENDIX D-4

CALCULATION OF MINIMUM MOLECULAR FORMULA AND AQ

Run 8
Light Input = 4.2 KW
Agitation Rate = 45 cycles/minute

Algae analysis

%C	= 51.29
%H	= 7.12
%N	= 10.79
% Ash	= 5.74
Total	= 74.94

%O = 100.00 - 74.94 = 25.06

To determine the molecular formula, divide the constituents by their atomic weight and reduce the resultant gram-atoms by the lowest common denominator.

$$C = \frac{51.29}{12.00} = 4.274$$

$$H = \frac{7.12}{1.00} = 7.120$$

$$N = \frac{10.79}{14.00} = 0.771$$

$$O = \frac{5.74}{16.00} = 1.566$$

Dividing by 0.771 you get:

C = 5.54

H = 9.23

N = 1

O = 2.03

Therefore, the molecular formula is $C_{5.54}\ H_{9.23}\ O_{2.03}\ N$. To determine the AQ, balance the following equation.

$$0.5(N_2H_4CO) + CO_2 + H_2O \longrightarrow C_{5.54}H_{9.23}O_{2.03}N + O_2$$

The balanced equation becomes:

$$0.5(N_2H_4CO) + 5.04CO_2 + 3.64H_2O \longrightarrow C_{5.54}H_{9.23}O_{2.03}N + 6.10\ O_2$$

and the AQ equals:

$$\frac{CO_2}{O_2} = \frac{5.04}{6.10} = 0.826$$

APPENDIX D-5

SAMPLE CALCULATION OF R-VALUE AND PERCENTAGE COMPOSITION

R-VALUE

Run 5

$$\text{R-Value} = \frac{(\%C \times 2.664 + \%H \times 7.936 - \%O)}{3.99}$$

%C = 49.21
%H = 7.61
%N = 9.37
Ash = 5.98
72.17 - 100 = 27.83 % oxygen

49.21 x 2.664 = 131.095
7.61 x 7.936 = 60.392
191.487
Subtract oxygen -27.83
163.657 x 100 = 16,365.7 / 398.9 = 41.02 = R-value

PERCENTAGE COMPOSITION

% Protein (P) x 42 + % Carbohydrate (C) x 28 + % Lipid (L) x 67.5 = R-value x 100

% P + % C + % L = 100%

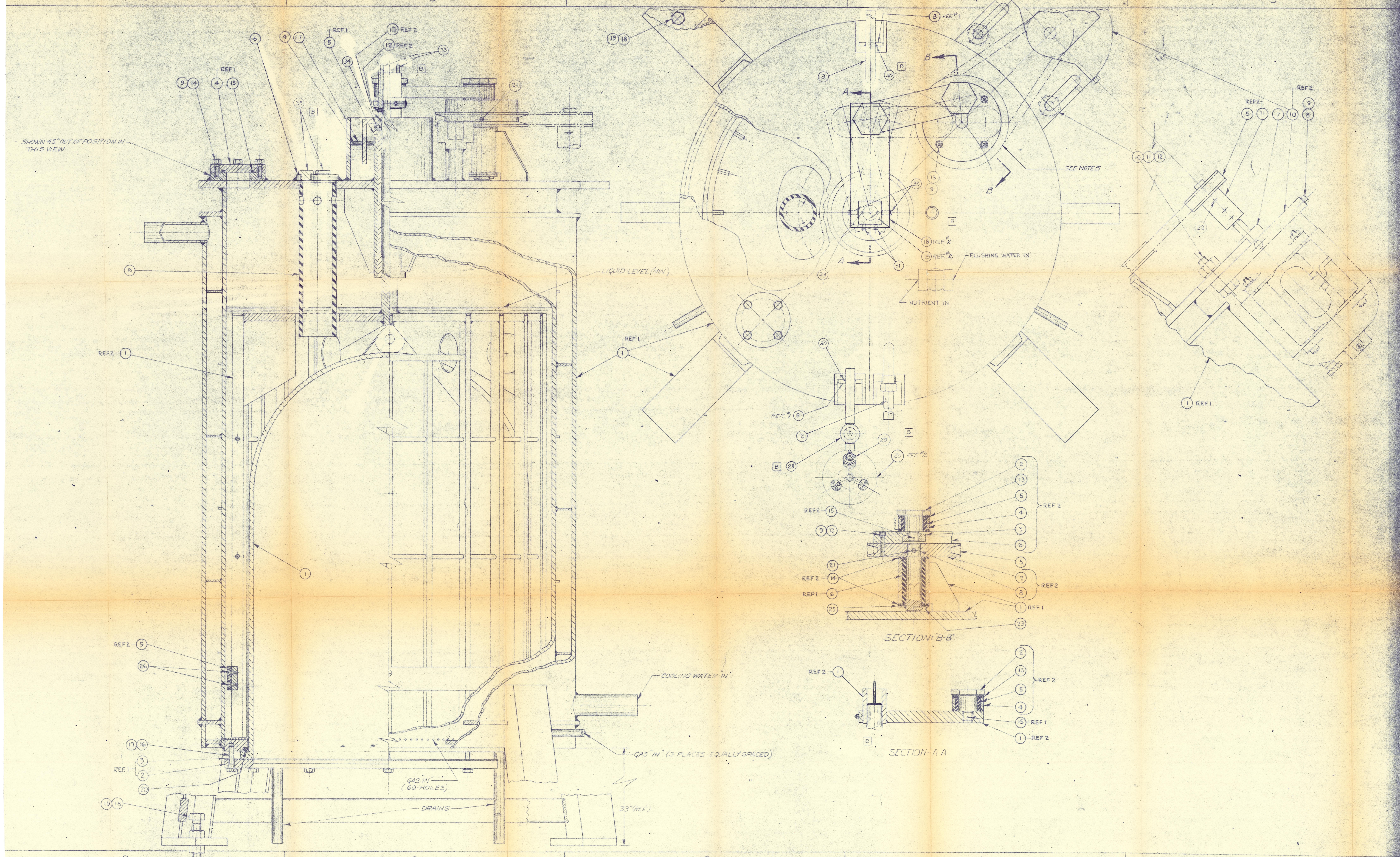
Protein = 9.37 x 6.25 = 58.6

(58.6) (42) + 28C + 67.5L = 4102

58.6 + C + L = 100

Carbohydrate = 29.1

Lipid = 12.3



	PC NO.	NAME	QUANTITY	MAT'L OR VDR'S DWG & CODE	SPEC	REMARKS	ZONE
	1	REACTION FLASK	1	PYREX		ORDERED ON P.O.# F-1123-922	
B	2	TEMP SWITCH (LAMP INTERLOCK)	1	SEE NOTE 1		EXISTING (TX-1)	
B	3	TEMP CONTROL SWITCH	1	SEE NOTE 1		EXISTING (TCX-)	
	4	MERCURY	40# (ORDER 80#)	SEE NOTE 5			
	5	V-BELT	1			EBCN 78-06-0710	
	6	LEVEL PROBE UNIT	1	SEE NOTE 1		EXISTING	
	7	MOTOR 1/4 HP	1	SEE NOTE 2		EXISTING	
	8	BOLT, HEX HD MACH 1/4"-20 UNC-2×1 1/4" LG	3	STEEL	MIL-B-857	TY III GR 2	
	9	WASHER, LOCK 1/4 SIZE	11	STEEL	FF-W-84	CL A STY 2	
	10	BOLT, HEX HD 5/8"-11 UNC-2×2 1/2" LG	2	STEEL	MIL-B-857	TY III GR 2	
	11	NUT, HEX 5/8"-11 UNC-2	2	STEEL	MIL-B-857	TY III GR 2	
	12	WASHER, LOCK 5/8 SIZE	2	STEEL	FF-W-84	CL A STY 2	
	13	SCREW, SOC CAP 1/4"-20 UNC-3×1" LG	4	STEEL	MIL-S-933	TY C	
	14	BOLT, HEX HD MACH 1/4"-20 UNC-2×7/8" LG	4	STEEL	MIL-B-857	TY III GR 2	
	15	O-RING AN6230B	1	BUNA	MIL-P-5516	CL B 1 5/8"×1 7/8"×1/8"	
	16	BOLT, HEX HD MACH 5/16"-18 UNC-2×1 1/2" LG	12	CRES	QQ-S-763	CL 304 COND B	
	17	WASHER, LOCK 5/16" SIZE	12	STEEL	FF-W-84	CL A STY 2	
	18	BOLT, HEX HD MACH 1/2"-13 UNC-2×2 1/2" LG	4	STEEL	MIL-B-857	TY III GR 2	
	19	NUT, HEX 1/2"-13 UNC-2	8	STEEL	MIL-B-857	TY III GR 2	
	20	O-RING (1/4" CROSS SECTION)	5 FT	BUNA	MIL-P-5516	CL B CUT TO SUIT	
	21	ROLL PIN 5/16"×2" LG	1	STEEL	MIL-P-10971	TY I COMP A	
	22	ROLL PIN 1/4"×1 1/2" LG	1	STEEL	MIL-P-10971	TY I COMP A	
	23	PIN, COTTER 3/32" DIA × 1 1/2" LG	1	CRES	FF-P-386	TY A	
B	~~24~~	~~PIN, COTTER 1/8" DIA × 1 1/2" LG~~	~~1~~	~~CRES~~	~~FF-P-386~~	~~TY A~~	
B	25	WASHER, PLAIN 1" SIZE 1/8 THK	1	STEEL	FF-W-92	TY A GR 1 CL A	
	26	SCREW, NYLOCK, FH. #10-32 × 1/2" LG	26	CRES	SEE NOTE 3		
	27	CLEAR MINERAL OIL	AS REQD	SEE NOTE 4			
B	28	VALVE, NEEDLE 1/4" NPT	1	CRES	COML	HOKE VALVE CO.	
B	29	LEVEL PROBE	3	SEE NOTE 6			
B	30	"O" RING AN 6227B-12	2	BUNA	MIL-P-5516	CL B 5/8"×13/16"×3/32"	
B	31	SCREW, SOC. HD. SET 1/4"-20 UNC-3A×3/8" LG	2	CRES	QQ-S-763 CL 303 TY G COND A	(CUP POINT)	
B	32	SCREW, SOC. HD. SET 1/4"-20 UNC-3A×5/8" LG	2	CRES	QQ-S-763 CL 303 TY G COND A	(CUP POINT)	
B	33	KEY, ROUND 3/16" DIA × 2" LONG	2	STEEL	AISI. W1-1.20C	(DRILL ROD)	
B	34	BEARING, THRUST	1	SEE NOTE #7			
B	35	PLUG, HEX 14mm-1.25mm Pitch	3	CRES	COML.		

GENERAL NOTES

1. PCS. #2 & 3 TO BE STORED IN DEPT 413
2. PC 7 TO BE STORED IN CHEM LAB, DEPT 411
3. PC 26 TO BE NYLOCK FLAT HEAD SCREW #10-32 × 1/2" LONG, CRES, AS DESCRIBED ON PAGE 12, CATALOG 58-A, NYLOCK CORP. 611 INDUSTRIAL AVE., PARAMUS, N.J. - OR EQUAL.
4. AVAILABLE FROM CHEM. ENG. LABORATORY.
5. PULLEY ARRANGEMENT SHOWN USED IN CONJUNCTION WITH A DOUBLE SHEAVE IDLER PULLEY. THIS DOES NOT CHANGE AGITATOR SPEED BUT ALLOWS MOTOR TO OPERATE AT HIGER SPEED OBTAINING BETTER TORQUE CHARACTERISTICS.
6. PC. #29 TO BE MODEL J-14-64C-2 SPARK PLUG LEVEL PROBE AS MANUFACTURED BY THE CHAMPION SPARK PLUG CO., TOLEDO 1, OHIO.
7. PC. #34 TO BE #FT-9 THRUST BEARING AS MANF. BY THE ANDREWS BEARING CO., SPARTANBURG, S.C.

REV	ZONE	DESCRIPTION	DATE	APPD	DATE	APPD
B	ALL	TO COVER FIELD TEST MODIFICATIONS				

REVISIONS

NO.	TITLE	MFR OR CONTR DRAWING No.
2	PHOTOSYNTHETIC GAS EXCHANGER UNIT - DETAILS - SHEET 2	H-3096
1	PHOTOSYNTHETIC GAS EXCHANGER UNIT - DETAILS - SHEET 1	H-3095

REFERENCES

H-3094

GENERAL DYNAMICS CORP
ELECTRIC BOAT DIVISION
GROTON, CONN.

DR G.S. G.K.

PHOTOSYNTHETIC GAS EXCHANGER UNIT ASSEMBLY

APPROVED

N.A.S.A.

SCALE 6"=1'-0"

REV. B